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Contribution of a Typing Method Based on IS*256* Probing of *Sma*I-Digested Cellular DNA to Discrimination of European Phage Type 77 Methicillin-Resistant *Staphylococcus aureus* Strains

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The incidence of infections with phage type 77 methicillin-resistant *Staphylococcus aureus* (MRSA) strains increased in France in 1987. These strains are widespread in numerous European hospitals. The *Sma*I restriction profiles of total DNA extracted from 74 phage type 77 MRSA strains isolated from 1987 to 1994 in 10 hospitals in eight European cities (in France, Belgium, and Spain) were analyzed. Hybridization with a probe containing a 468-bp DNA fragment from within the transposase gene of the insertion sequence IS*256* was also examined. Forty-three *Sma*I profiles were detected. Twenty major genotypes were identified, and each genotype contained strains with the same profile or profiles which differed by no more than three bands. Strains isolated in different countries and at several-year intervals were often grouped within the same genotype. A larger number of genotypes could be discriminated by analysis of the patterns of hybridization with the IS*256* probe. *Sma*I restriction fragments with the same apparent electrophoretic mobility could, in some cases, be distinguished by the presence or the absence of nucleotide sequences hybridizing with IS*256*. The strains that grouped within the same genotype after hybridization with IS*256* were mostly those isolated in the same hospital and at less than 12-month intervals. Consequently, the IS*256* probe that we used improved restriction profile analysis for discrimination between the intrahospital, outbreak-related phage type 77 MRSA strains and the endemic strains disseminated in various cities and countries.

The so-called phage type 77 methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been isolated in several French hospitals since December 1984 (1, 16, 28). The increased incidence of these strains was shown to coincide, in a Parisian hospital, with that of resistance to fluoroquinolones among staphylococci (1). Phage type 77 MRSA strains are resistant to numerous antibiotics including β-lactams and fluoroquinolones and are susceptible to phage 77 at the routine test dilution (RTD). Susceptibility to other phages of the international basic set (4), including 54, 83A, 75, and/or 84, is occasionally detected, usually when phages are used at 100 RTDs and/or after incubation of bacterial cultures at 56°C for 2 min (28). A significant increase in the prevalence of such strains was observed in France between 1985 and 1987 (1, 16, 28), and MRSA strains belonging to the same phage type were also detected in Spain (2, 40), Portugal, Belgium (36), Germany (44, 46), and the United Kingdom (8).

*Eco*RI-digested cellular DNAs from 48 such strains isolated between 1984 and 1991 in seven French hospitals and one Spanish hospital have been typed with the IS*256* probes pIP1307 (28) and pIP1551 (15). The genomes of most of these phage type 77 European MRSA strains were more closely related to each other than to independent MRSA strains belonging to different phage types (16, 28).

In this study, phage type 77 MRSA strains isolated between 1987 and 1994 in 10 hospitals in eight cities in Europe (in France, Belgium, and Spain) were compared by analysis of the *Sma*I restriction patterns before and after hybridization with an IS*256* probe, pIP1551 (15). The purpose of this work was to evaluate the discriminatory power of the two typing methods by using the same criteria to cluster the strains into genotypes or subtypes.

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MATERIALS AND METHODS

Bacterial strains and plasmids. A total of 88 MRSA strains originating from different infected individual patients were studied (see Table 1). By using the international set of *S. aureus* phages (4) at the RTD, 74 strains were identified as belonging to the same phage type, named phage type 77, because they were susceptible either only to phage 77 (50 strains) or to phage 77 and to one or two additional phages (phages 54, 83A, 54 and 83A, or 83A and 85), with one of the two additional phages exhibiting weak reaction (24 strains). Nine strains were susceptible to phage 77 and exhibited a strong reaction with at least three additional phages and thus belonged to different phage types. Five strains were not typeable, despite the use of phages at 100 times the RTD and were included because they were isolated in the same locations and during the same time periods as the phage type 77 strains.

The strains were isolated between 1987 and 1994 in 10 hospitals in eight cities in Europe (in France, Belgium, and Spain). The 17 strains isolated in 1987 in one Parisian hospital, hospital Br (France) (1), and the nine strains isolated in 1993 in the hospital of Bordeaux, hospital Bo (France) (Table 1), were collected during intrahospital outbreaks. The other strains were from hospitals in which such strains had been endemic for several years, with no evidence suggesting an early outbreak.

Plasmid pIP1551 (15) was used as a probe. It consisted of pUC18 carrying a 468-bp insert from the transposase gene of the insertion sequence IS*256* (5).

Susceptibilities to antibiotics. Susceptibilities to antibiotics were determined by a disk diffusion assay (6) with commercially available antibiotic disks (Diagnostics Pasteur). Additional disks prepared in our laboratory contained pristinamycin IIA (20 μg) or pristinamycin IB (40 μg).

PFGE. The protocol for the preparation and *Sma*I cleavage of cellular DNA was as described previously (12). The restriction fragments were separated by pulsed-field gel electrophoresis (PFGE) in a CHEF Pulsaphor system (Pharmacia, LKB Biotechnology). Plugs containing the digested DNA were inserted into a 1.2% (wt/vol) agarose gel, and electrophoresis was carried out in TBE buffer (50 mM Tris base, 50 mM boric acid, 20 mM EDTA [pH 7.5]) at 12°C with alternating pulses at an angle of 120°. The *Sma*I-digested DNA was separated at 235 V with a 5-s pulse time for 4 h, a 15-s pulse time for 6 h, a 25-s pulse time for 8 h, and a 35-s pulse time for 9 h. Bacteriophage lambda DNA concatemers (Bio-Rad) and *Sma*I-digested DNA of a study MRSA strain defined as having *Sma*I pattern 1 (see Table 1 and Fig. 1A to C, lanes 13) were used as size standards.

Blotting and hybridization. DNA was transferred from agarose gels to Hybond N^+ membranes (Amersham International) with a Trans-Vac TE80 vacuum blotter (Hoefer Scientific Instruments, San Francisco, Calif.). Prehybridization and hybridization were performed under stringent conditions as described previously (7). The plasmid used as a probe was labeled with [α-^{32}P]dCTP (110 TBq · $mmol^{-1}$) by random priming by using the Megaprime DNA labeling system (Amersham International). The blots were exposed to Hyperfilm (Amersham) at −80°C.

Data analysis. Macrorestriction fingerprints were compared visually. All isolates were analyzed in three or more runs to allow for the migration of DNA samples with similar patterns in adjacent lanes and thus to ascertain pattern relatedness better. The sizes of the *Sma*I restriction fragments and the relevant characteristics of the studied strains were entered into a database (Sybase). Dice coefficients of similarity (13) were determined for each pair of strains. Clustering was assessed by the unweighted pair group method with arithmetic averages.

Each *Sma*I pattern was compared to pattern 1 (see Table 1), and the number of band differences was evaluated. The strains were clustered into three categories named closely related, possibly related, or different, according to the criteria proposed by Tenover et al. (39). Among the closely related strains, genotype A was assigned to the strains with pattern 1. Strains with patterns that differed by one to three *Sma*I bands were clustered into subtypes designated by the same letter (A), with numeral suffixes. Within each of the two remaining categories, the strains were distributed into subtypes and major genotypes by comparing each *Sma*I pattern to the predominant pattern of each category.

Within each major *Sma*I genotype, the *Sma*I patterns were also compared by analysis of the differences revealed by hybridization with an IS*256* probe. Any two *Sma*I bands having the same apparent mobility differing by the presence or absence of nucleotide sequences hybridizing with the probe were assigned different sizes in the database, thus yielding a difference of two bands between the two patterns. Despite the greater genomic polymorphism revealed after hybridization with the IS*256* probe, the criteria used to cluster the strains into major genotypes and subtypes were the same as those used after analysis of *Sma*I patterns. Thus, the strains having the same *Sma*I pattern before and after hybridization with the IS*256* probe belong to the same subtype and the patterns of the strains clustered in the same major genotype differ by three or fewer bands (bands of different sizes or distinguishable by whether or not they hybridize with IS*256*). The genotypes characterized by IS*256* probing are designated by roman numerals, and the subtypes are designated by roman numerals with letter suffixes.

RESULTS

Analysis of the antibiotypes. The strains studied had 10 antibiotic resistance markers in common (Table 1). The number of additional markers was between zero and six, depending on the strain. The strains belonging to the different phage types and those which were not phage typeable were not distinguishable by antibiotyping.

Analysis of the *Sma*I patterns. The number of *Sma*I fragments in the pattern of each strain was from 15 to 19. The patterns of 17 phage type 77 strains are shown in Fig. 1. A total of 43 different patterns was detected among the 88 strains tested (Table 1).

Eleven of the 17 phage type 77 and outbreak-related MRSA strains isolated in 1987 in hospital Br (France) (1) had the same pattern, pattern 1 (Table 1). Strains giving this pattern were assigned to genotype A, and all the other patterns were compared to pattern 1. In addition to the markers common to all the tested strains, 7 of the 12 strains having pattern 1 were resistant to rifampin and five were resistant to rifampin and fosfomycin (Table 1).

Patterns 2 to 14 (Table 1) differed from pattern 1 by no more than three *Sma*I fragments. The 32 closely related strains having patterns 2 to 14 were distributed into 13 genomic subtypes (subtypes A1 to A13). They included strains isolated from 1987 to 1994 in eight hospitals in eight European cities (five in France, two in Belgium, and one in Spain). Twenty-eight of these strains belonged to phage type 77, two strains belonged to a different phage type, including a strong reaction with phage 77, and two strains were not phage typeable. Each *Sma*I subtype contained one to seven strains. The strains grouped in the same subtype were either isolated in the same hospital during the same year (subtypes A3, A4, and A5) or in hospitals located in different cities and countries and at time intervals of 4 or 5 years (subtype A9, 1991 to 1994; subtype A10, 1987 to 1991).

*Sma*I patterns 15 to 23 differed from pattern 1 by four to six bands. According to the criteria proposed by Tenover et al. (39), the 18 strains having these patterns may be clustered into the category of possibly related strains. Six of these strains isolated in Aalst (Belgium) from 1990 to 1992 gave the same pattern, pattern 15 (genotype B). By comparison with pattern 15, the 12 other strains of this category were distributed into four subtypes (subtypes B1 to B4, eight strains) and four different genotypes (genotypes C to F, four strains).

The 26 strains having patterns 24 to 43 were considered to be different from those having pattern 1 because the number of fragment differences was at least seven. The strains in this category were distributed into 14 major genotypes (genotypes G to T); genotype G included 10 strains.

Each of the three categories (closely related to strains having pattern 1, possibly related, or different) included strains from various sources, dates of isolation, or phage types, except that all strains isolated in 1987 fell into the category of closely related strains (major genotype A: A, A1, A3, A7, and A10). For the strains isolated in Bordeaux (France) in 1993 and suspected of being outbreak related because MRSA strains were not detected in this hospital before 1993, there was a relatively good correlation between the modes of clustering based on phage typing and on analysis of *Sma*I patterns. Indeed, the three strains susceptible to several phages were grouped in the category of different strains (genotype K), whereas those which were either weakly susceptible to phages 77, 83A, and 85 or not typeable were related (subtype A9, one strain) or possibly related (subtypes B2 and B3, five strains). These results suggest that the MRSA strains isolated in Bordeaux originated from two or three different sources.

A dendrogram was constructed on the basis of the levels of similarity of the 43 *Sma*I patterns detected for the 88 strains tested (Fig. 2). The amalgamation distance for the patterns with at least 81% similarity (i.e., difference in the *Sma*I patterns involving fewer than six fragments) varied from 0 to 25%. Two of the five similarity clusters included strains belonging to the categories of related strains (major genotype A) and possibly related strains (major genotypes B, C, D, E), but did not include any of the strains in the different strain categories (Fig. 2).

Analysis of *Sma*I patterns after hybridization with IS*256* probe. *Sma*I digests of the DNAs from strains grouped in the same major genotype were tested for hybridization with the IS*256* probe. The results obtained for 17 phage type 77 strains clustered in major genotype A are reported in Fig. 1. In some cases, *Sma*I fragments with indistinguishable mobilities in different patterns could be discriminated by the presence or absence of nucleotide sequences hybridizing with IS*256*. For example, the patterns in lanes 13 and 14 differ from those in the other lanes by 6 to 16 bands after hybridization with the IS*256* probe, whereas there were fewer than two *Sma*I band differences in the total DNA banding patterns. Thus, the 44 strains that grouped in the same *Sma*I major genotype (genotype A, subtypes A1 to A13) were classified into 22 different genotypes by IS*256* hybridization (Table 1). Hybridization with IS*256* also discriminated among the strains belonging to genotypes B (genotype B, subtypes B1 to B4) and G (genotype G, subtypes G1 to G6), whereas the four strains of genotype K remained clustered in the same *Sma*I and IS*256* genotype (XXXVIII and XXXVIIIa). With this increase in the discriminatory power, the phage type 77 strains that grouped in the same *Sma*I and IS*256* genotype were mostly those isolated in the same hospital and within a period of less than 12 months (genotypes I, III, V,

TABLE 1. Relevant characteristics of the 88 MRSA strains analyzed

Comparison	*Sma*I pattern		*Sma*I and IS*256* genotype[b]	Date of isolation (mo/yr)	Source hospital, city, country	Antibiotic resistance in addition to the common markers[c]	Phage type (RTD)[d]
	No. of isolates	Genotype[a]					
Compare genotype to pattern 1 and *Sma*I-IS*256* genotype to I							
	1	A	I	1987	Hd, Paris, France	Rf	77++
	1	A	I	01/1987	Br, Paris, France	Rf Fm	77++
	1	A	I	01/1987	Br, Paris, France	Rf Fm	77++
	1	A	I	03/1987	Br, Paris, France	Rf Fm	77++
	1	A	I	04/1987	Br, Paris, France	Rf Fm	77++
	1	A	I	1987	Br, Paris, France	Rf Fm	77++
	1	A	Ia	01/1987	Br, Paris, France	Rf	77++
	1	A	Ib	07/1987	Br, Paris, France	Rf	77++
	1	A	Ib	10/1987	Br, Paris, France	Rf	77++
	1	A	Ib	10/1987	Br, Paris, France	Rf	77++
	1	A	Ib	11/1987	Br, Paris, France	Rf	77++
	1	A	Ib	12/1987	Br, Paris, France	Rf	77++
	2	A1	Ic	06/1987	Br, Paris, France	Rf	77++
	3	A2	II	05/1991	Br, Paris, France	Nm SgA Tp Rf	77±
	4	A3	III	02/1987	Br, Paris, France	None	77+
	4	A3	III	03/1987	Br, Paris, France	Nm	77+
	4	A3	III	11/1987	Br, Paris, France	Nm	77+
	4	A3	III	11/1987	Br, Paris, France	Nm	77+
	5	A4	IV	05/1993	To, Toulouse, France	Nm SgA Tp Rf Fa Fm	77±
	5	A4	V	05/1993	To, Toulouse, France	Nm SgA Tp Rf Fa Fm	77±
	5	A4	V	05/1993	To, Toulouse, France	Nm SgA Tp Rf Fa Fm	77±
	5	A4	V	09/1993	To, Toulouse, France	Nm SgA Tp Rf Fa Fm	77±
	6	A5	VI	05/1993	To, Toulouse, France	Nm SgA Tp Rf Fa Fm	77±
	6	A5	VI	05/1993	To, Toulouse, France	Nm SgA Tp Rf Fa Fm	NT
	7	A6	VII	07/1993	To, Toulouse, France	Nm SgA Tp Rf Fa Fm	77+, 75+, 83A+, 85+
	8	A7	VIII	1987	To, Toulouse, France	Nm SgA Tp Rf Fa Fm	77++
	9	A8	IX	12/1992	Aa, Aalst, Belgium	None	77++, 54+, 83A+
	9	A8	X	08/1993	Aa, Aalst, Belgium	Nm	77++, 54++, 83A+
	10	A9	XI	11/1993	Bo, Bordeaux, France	Nm Rf Fa Fm	NT
	10	A9	XII	04/1991	Br, Paris, France	Nm SgA Tp Rf Fm	77±
	10	A9	XII	05/1991	Br, Paris, France	Nm SgA Tp Rf Fm	77++
	10	A9	XII	12/1994	Gh, Ghent, Belgium	Nm	77++, 83A±
	10	A9	XII	12/1994	Gh, Ghent, Belgium	Nm	77++, 83A±
	11	A10	XIII	1991	Sj, Paris, France	Nm Rf Fm	77+
	11	A10	XIII	1991	Sj, Paris, France	Nm Rf Fm	77++
	11	A10	XIV	10/1989	Ba, Barcelona, Spain	Nm Rf	77±
	11	A10	XV	10/1989	Ba, Barcelona, Spain	Nm Rf	77±
	11	A10	XVI	1987	Di, Paris, France	None	77++
	11	A10	XVII	1987	Br, Paris, France	Nm Rf	77++
	11	A10	XVIII	1991	Sj, Paris, France	Nm Rf Fm	77±
	12	A11	XIX	03/1992	Gh, Ghent, Belgium	Rf	77++, 47++, 54++, 75++, 85++
	13	A12	XX	10/1989	Ba, Barcelona, Spain	Nm Rf	77++, 54++, 75++, 84++, 85++
	13	A12	XXI	1991	Sj, Paris, France	Nm Rf	77±
	14	A13	XXII	1991	Sj, Paris, France	Nm Rf Fm	77++
Compare genotype to pattern 15 and *Sma*I-IS*256* genotype to XXIII							
	15	B●	XXIII	01/1992	Aa, Aalst, Belgium	None	77++, 54++, 83A+
	15	B●	XXIII	12/1991	Aa, Aalst, Belgium	None	77++, 54++, 83A+
	15	B●	XXIIIa	11/1990	Aa, Aalst, Belgium	Fm	77++, 54++, 83A++

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TABLE 1—*Continued*

Comparison	*Sma*I pattern		*Sma*I and IS*256* genotype[b]	Date of isolation (mo/yr)	Source hospital, city, country	Antibiotic resistance in addition to the common markers[c]	Phage type (RTD)[d]
	No. of isolates	Genotype[a]					
	15	B●	XXIIIb	11/1990	Aa, Aalst, Belgium	None	77++, 54++, 83A++
	15	B●	XXIIIc	11/1990	Aa, Aalst, Belgium	None	77++, 54++, 83A+
	15	B●	XXIV	02/1991	Aa, Aalst, Belgium	Fm	77++, 54++
	16	B1●	XXV	04/1992	Gh, Ghent, Belgium	None	77++, 47++, 54++, 75++, 85++
	16	B1●	XXV	1993	Br, Paris, France	Nm SgA Tp Rf Fa Fm	77+
	17	B2●	XXVI	11/1993	Bo, Bordeaux, France	Nm Rf Fa Fm	77+
	17	B2●	XXVI	11/1993	Bo, Bordeaux, France	Nm Rf Fa Fm	77±, 83A±, 85±
	17	B2●	XXVI	11/1993	Bo, Bordeaux, France	Nm Rf Fa Fm	NT
	17	B2●	XXVI	11/1993	Bo, Bordeaux, France	Nm Rf Fa Fm	NT
	18	B3●	XXVII	11/1993	Bo, Bordeaux, France	Nm Rf Fa Fm	NT
	19	B4●	XXVIII	1993	Br, Paris, France	Nm SgA Tp Rf Fm	77++
	20	C●		05/1991	Br, Paris, France	Nm SgA Tp Rf Fa Fm	77++
	21	D●		1990	Go, Gonesse, France	Nm Rf	77+
	22	E●		10/1989	Ba, Barcelona, Spain	Nm Rf	77±
	23	F●		06/1992	Gh, Ghent, Belgium	None	77++
Compare genotype to pattern 24 and *Sma*I-IS*256* genotype to XXIX							
	24	G	XXIX	02/1992	Aa, Aalst, Belgium	None	77++, 54++, 83A+
	24	G	XXIX	04/1992	Aa, Aalst, Belgium	None	77++, 54++, 83A+
	24	G	XXX	10/1989	Ba, Barcelona, Spain	Nm Rf	77±, 54++, 83A++, 84++, 85++
	25	G1	XXXI	11/1993	Aa, Aalst, Belgium	None	77++, 54++, 83A+
	26	G2	XXXII	10/1993	Aa, Aalst, Belgium	Nm	77++, 54++, 83A±
	26	G2	XXXIII	12/1994	Gh, Ghent, Belgium	None	77++, 54++
	27	G3	XXXIV	12/1994	Gh, Ghent, Belgium	Nm	77++, 54++, 83A+
	28	G4	XXXV	02/1992	Gh, Ghent, Belgium	Rf	77++, 54++, 83A±
	29	G5	XXXVI	06/1992	Aa, Aalst, Belgium	None	77++, 54++
	30	G6	XXXVII	10/1989	Ba, Barcelona, Spain	Nm Rf	77++, 54++, 75++, 84++, 85++
	31	H		12/1994	Aa, Aalst, Belgium	None	77++, 54++, 83A+
	32	I		09/1993	To, Toulouse, France	Nm SgA Tp Rf Fa Fm	77±
	33	J		03/1993	Gh, Ghent, Belgium	Nm	77++, 83A±
Compare genotype *Sma*I-IS*256* to XXXVIII							
	34	K	XXXVIII	11/1993	Bo, Bordeaux, France	Nm Rf Fa Fm	77+, 47+, 53±, 75+, 83A±, 85+
	34	K	XXXVIII	11/1993	Bo, Bordeaux, France	Nm Rf Fa Fm	77±, 47+, 53±, 54++, 75++, 83A++, 85++
	34	K	XXXVIII	11/1993	Bo, Bordeaux, France	Nm Rf Fa Fm	77±, 47++, 53++, 75++, 83A+, 85++
	34	K	XXXVIIIa	01/1993	Gh, Ghent, Belgium	None	77++, 54++, 83A+
	35	L		04/1993	Gh, Ghent, Belgium	Nm	77++
	36	M		1991	Br, Paris, France	Nm SgA Rf Fm	77+

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TABLE 1—*Continued*

Comparison	*Sma*I pattern		*Sma*I and IS*256* genotype[b]	Date of isolation (mo/yr)	Source hospital, city, country	Antibiotic resistance in addition to the common markers[c]	Phage type (RTD)[d]
	No. of isolates	Genotype[a]					
	37	N		05/1991	Br, Paris, France	Nm SgA Rf Fm	77±
	38	O		12/1994	Gh, Ghent, Belgium	None	77++, 54+, 83A+
	39	P		1993	Br, Paris, France	Nm SgA Tp Rf Fm	77±
	40	Q*		02/1993	Gh, Ghent, Belgium	None	77++
	41	R*		04/1993	Gh, Ghent, Belgium	Nm	77++
	42	S*		01/1992	Gh, Ghent, Belgium	Rf	77++
	43	T*		07/1993	Aa, Aalst, Belgium	None	77±, 54++

[a] Genotype A was assigned to strains with pattern 1 (Fig. 1). Because pattern 1 was predominant among the outbreak-related phage type 77 strains isolated in 1987 in the Parisian hospital Br, each of the remaining *Sma*I patterns was compared to pattern 1. Strains having patterns that differed from pattern 1 by one to three *Sma*I bands were considered closely related to A type strains and therefore clustered into subtypes designated A1 to A13. The strains belonging to the same subtype have the same pattern. The remaining strains were distributed into the following two categories: (i) those which may be considered possibly related to A type strains because they have patterns which differed from pattern 1 by 4 to 6 bands (genotypes B to F with dots) and (ii) those which were considered unrelated to A type strains because their patterns differed from pattern 1 by at least seven bands (genotypes G to T). Within each of these two categories, the strains were clustered by comparing their patterns to pattern 15 (genotype B) or pattern 24 (genotype G). Patterns 40 to 43 (genotypes Q to T, indicated by asterisks) differed between each other and from all the remaining patterns by at least seven fragments.

[b] The *Sma*I patterns including differences revealed after hybridization with the IS*256* probe were also compared. Within each of the *Sma*I genotypes A, B, G, or K, the *Sma*I-IS*256* patterns were compared to the predominant pattern, i.e., *Sma*I-IS*256* genotypes I, XXIII, XXIX, or XXXVIII, respectively. The subtypes of each of these latter genotypes are designated by the same roman numeral with letter suffixes.

[c] The markers shared by all the strains are resistances to the following antibiotics: β-lactams (penicillinase production and intrinsic resistance), fluoroquinolones including pefloxacin; tetracycline; minocycline; macrolides, lincosamides, and streptogramin B (constitutive resistance to MLS); sulfonamides; spectinomycin; streptomycin; and aminoglycosides modified by the bifunctional enzyme AAC6′-APH2″, including kanamycin, tobramycin, and gentamicin. Abbreviations: Fa, fusidic acid; Fm, fosfomycin; Nm, neomycin; Rf, rifampin; SgA, streptogramin A (MICs, ≥8 μg/ml); Tp, trimethoprim.

[d] The international set of 23 *S. aureus* phages were used at the RTD for phage typing (4).

VI, XIII, XXIII, XXVI, and XXIX; Table 1). However, identical patterns were occasionally observed among the phage type 77 strains isolated in different countries and at intervals of up to 44 months (genotypes XII and XXV).

The 17 phage type 77 strains isolated in the same Parisian hospital (hospital Br) in 1987 were distributed into three *Sma*I and IS*256* genotypes, genotypes I (12 strains), III (four strains), and XVII (one strain), whereas after analysis of *Sma*I patterns and by using the same criteria for defining types, all of these strains clustered within the same major genotype.

DISCUSSION

Although collected in three European countries and at time intervals of up to 8 years, a large number of the phage type 77 MRSA strains tested appeared to be closely related according to analysis of the *Sma*I macrorestriction patterns. Indeed, 40 of the 75 tested strains (53%) with this phage type clustered within the same major *Sma*I genotype (genotype A, subtypes A1 to A13). This major *Sma*I genotype also included MRSA strains of different phage types and some which were not typeable. The wide dissemination and long persistence of MRSA strains belonging to the same *Sma*I genotype has been observed by several investigators (9, 10, 14, 22–24, 29, 32–34, 36, 37, 43, 45). Additional typing methods such as plasmid analysis and hybridization with various probes have been proposed for identifying subclones among MRSA strains grouped in the same major *Sma*I genotype (9, 10, 14, 18, 20, 25, 26, 31, 33, 35, 37, 38).

Plasmid analysis was not appropriate for improved discrimination between phage type 77 MRSA strains isolated in France: 80% of the epidemiologically unrelated MRSA strains isolated in France carry a penicillinase plasmid with the same *Eco*RI pattern (19). Moreover, the plasmid harbored by one of these strains underwent rearrangements in a hot-spot region located close to the genes *bin* and *sin* encoding resolvases (11).

All gentamicin-resistant MRSA strains tested, independent of geographical origin, carry several copies of the insertion sequence IS*256* (15–17, 19, 28). In addition to the two copies of IS*256* which delimit transposon Tn*4001* (5, 30) carrying the gene *aacA-aphD* conferring resistance to gentamicin, kanamycin, and related aminoglycosides, most European strains carry several copies of IS*256* dispersed across various sites of the chromosome. Analysis of the IS*256* probe hybridization patterns with *Eco*RI or *Hin*dIII digests of total DNA has been shown to be a good method for discriminating gentamicin-resistant staphylococci (16, 27, 28, 41), and this typing method has been used in various laboratories (3, 42). In the present study, we have shown that the IS*256* probe revealed more differences than the numbers of differences observed by analysis of *Sma*I macrorestriction patterns alone.

*Eco*RI-digested total DNA from 48 of the strains included in this work has previously been tested with IS*256* probes (16, 28). Despite the use of several DNA preparations extracted from independent subcultures of the same strain, reproducible IS*256* hybridization patterns were observed, suggesting that intrastrain transposition events are not frequent. Therefore, such events probably have a minor contribution to the genomic heterogeneity revealed by IS*256*. The differences revealed by IS*256* between *Sma*I restriction fragments with indistinguishable electrophoretic mobilities may not necessarily be due only to the presence or the absence of nucleotide sequences hybridizing with the IS*256* probe but may also be associated with other sequence differences. It may therefore be possible to reveal these differences by comparing the restriction patterns obtained with several restriction enzymes instead of one or by using other probes.

Since the purpose of this study was to compare the classifi-

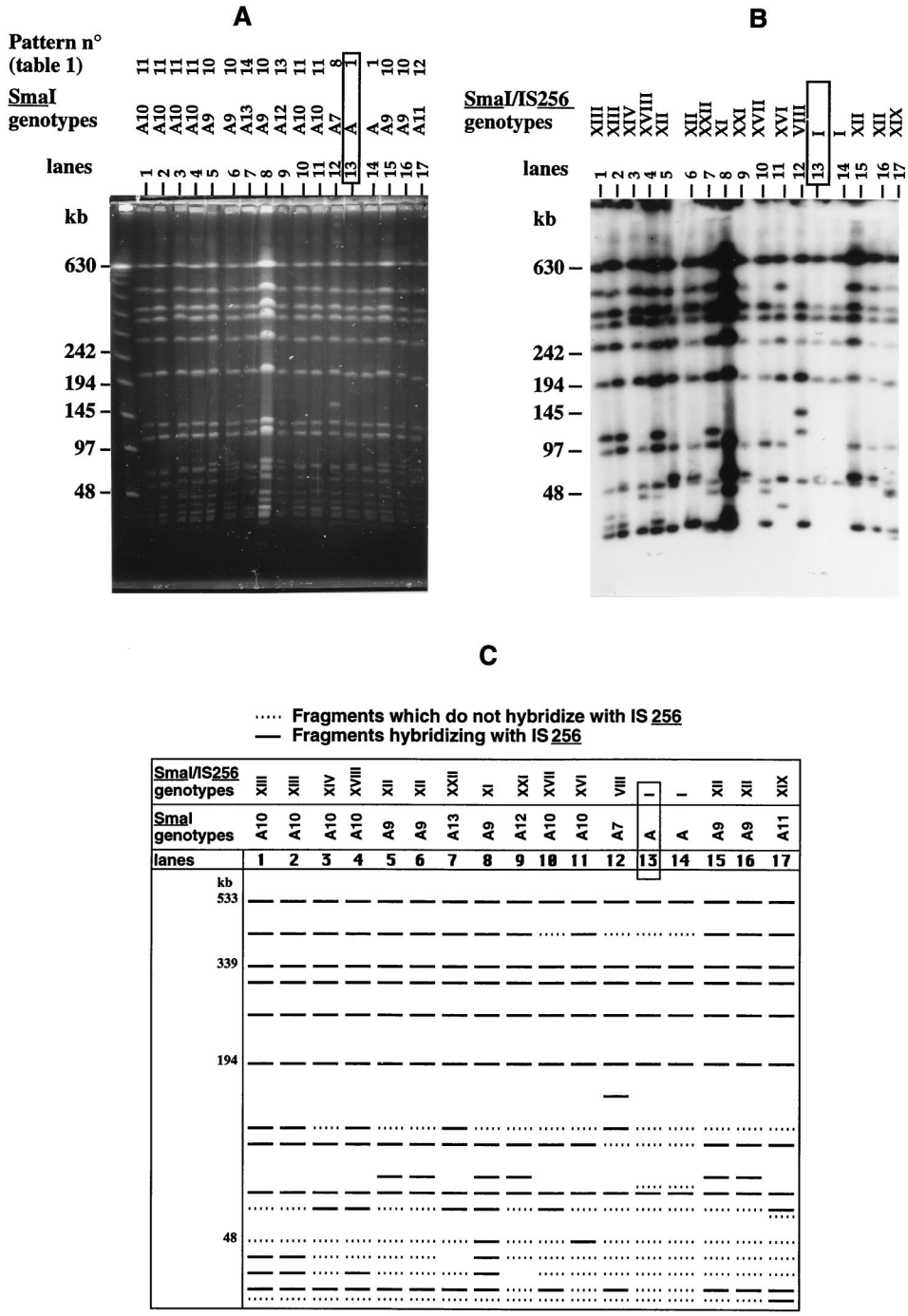

FIG. 1. PFGE of *Sma*I-digested total DNA from 17 phage type 77 MRSA strains. (A) *Sma*I macrorestriction patterns; (B) hybridization patterns with IS*256* probe; (C) schematic representation of the patterns.

cations into major genotypes or subtypes of the strains typed according to two markers, i.e., *Sma*I or *Sma*I and IS*256* patterns, we used the same criteria in both approaches to evaluate the relatedness of the strains (39). The advantage of the criteria proposed by Tenover et al. (39) is that they are based on the number of genetic events resulting in band pattern changes, independently of the technique used to reveal such changes. In the present study, major *Sma*I and IS*256* genotypes included, in most cases, strains isolated in the same hospital and within a period of less than 12 months. Thus, the probability that the strains are associated with the same intrahospital outbreak is higher if they are clustered within the same genotype by analysis of *Sma*I and IS*256* patterns than by analysis of *Sma*I patterns. However, the fact that strains belong to the same *Sma*I and IS*256* genotype does not exclude the possibility that they are derivatives of an endemic clone which is particularly stable. Indeed, two of the *Sma*I and IS*256* genotypes characterized in this study (genotypes XII and XXV; Table 1) con-

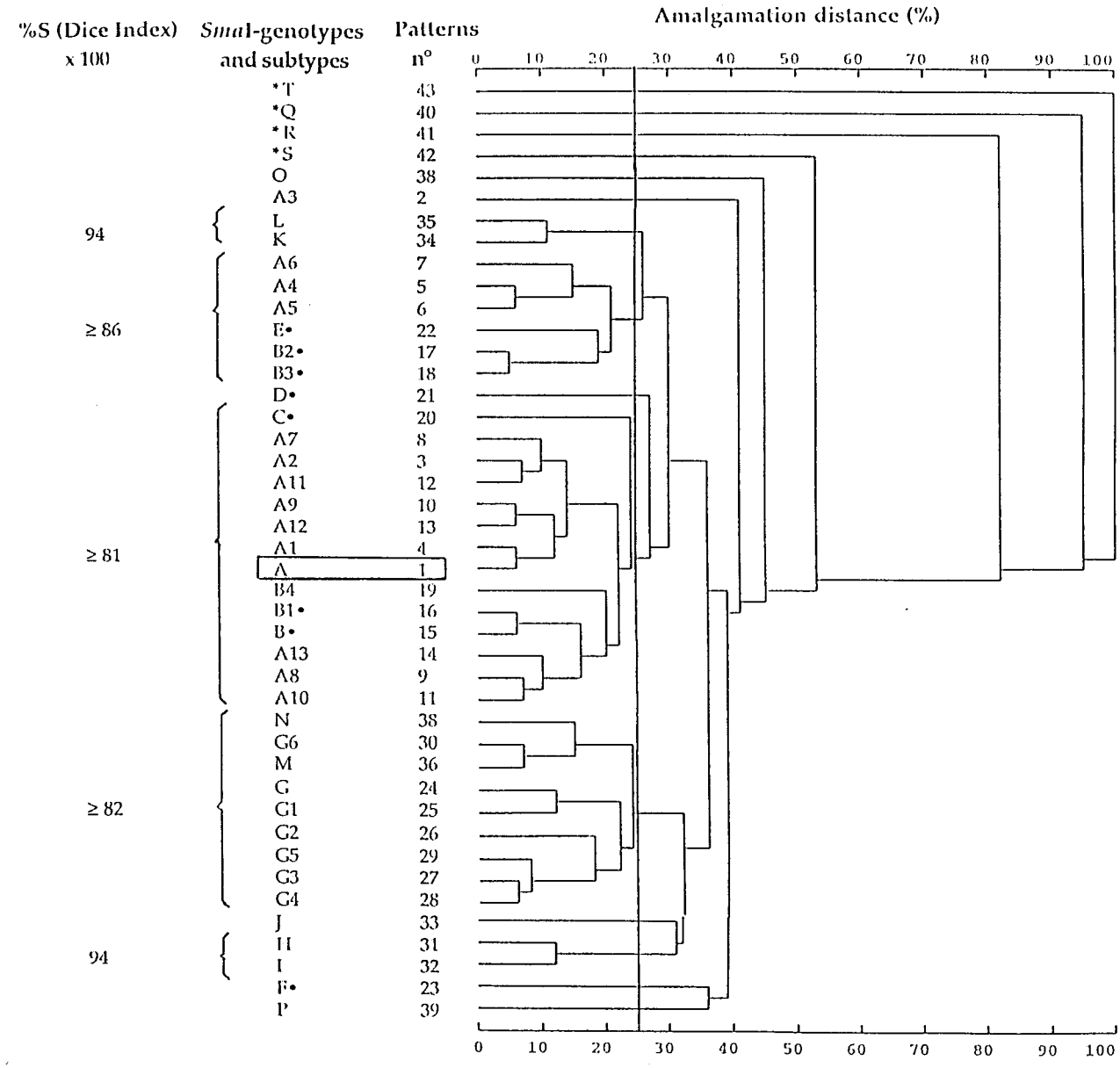

FIG. 2. Classification of the 43 *Sma*I patterns obtained for the 88 MRSA strains investigated (Table 1). Similarity between patterns was measured by using the Dice coefficient. Clustering was performed by an unweighted pair-group method with arithmetic averages. ●, possibly related to pattern 1 (Table 1); ∗, unrelated to each other and to all other remaining strains.

tained strains from independent sources and isolated at time intervals of up to 44 months. Several factors may contribute to the persistence of endemic clones. They include not only the expression of virulence factors (21) and resistance to environmental pressures but also the stability of the genome.

The discriminatory power required for strain typing depends on the questions addressed. A highly discriminative typing method is useful for investigation whether the recent increase in staphylococcal infections in hospitals is due to staphylococcal strains derived from previously present clones which were supposed to be eradicated by control measures or from clones recently introduced by colonized patients. Good discrimination is also required to compare strains isolated from the same patient.

Improving discriminatory power requires more stringent criteria for defining types or subtypes. However, such criteria cannot be proposed in terms of a degree of relatedness under which the strains may be clustered in the same type or subtype, because this depends partly on the stability of the marker, which is not necessarily the same for all strains. Therefore, none of the typing methods can be considered the best in all situations.

The discrepancies, resulting from the use of two molecular typing methods, between the mode of distribution in types of the 17 phage type 77, pefloxacin-resistant (Pf^r) MRSA strains isolated in 1987 in hospital Br (Table 1) could not be elucidated because no information concerning the probable source of these strains was available (1). It was shown that Pf^r MRSA strains were nosocomially acquired by patients whether or not they were treated with pefloxacin within an interval of 2 to 90

days following admission to the hospital. For one patient, infected with a Pf^s MRSA strain, the phage type 77 Pf^r MRSA strain selected by the treatment was suspected of being a mutant derivative of the infecting strain because both strains belonged to the same phage type. Moreover, phage type 77 Pf^s MRSA strains had been detected in this hospital in 1987, but their incidence, their putative source(s), and the degree of genomic polymorphism among these strains were unknown. In particular, there are no data indicating whether the patients treated with pefloxacin were colonized with Pf^s phage-type 77 acquired in another hospital before their hospital admission relative to the present study.

In conclusion, satisfactory interpretation of bacterial typing results requires not only the use of several methods for good discrimination but also the assistance of infection control personnel to provide extensive epidemiological data.

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