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Molecular Characterization of *Staphylococcus sciuri* Strains Isolated from Humans

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We previously characterized over 100 *Staphylococcus sciuri* isolates, mainly of animal origin, and found that they all carried a genetic element (*S. sciuri mecA*) closely related to the *mecA* gene of methicillin-resistant *Staphylococcus aureus* (MRSA) strains. We also found a few isolates that carried a second copy of the gene, identical to MRSA *mecA*. In this work, we analyzed a collection of 28 *S. sciuri* strains isolated from both healthy and hospitalized individuals. This was a relatively heterogeneous group, as inferred from the different sources, places, and dates of isolation and as confirmed by pulsed-field gel electrophoresis analysis. All strains carried the *S. sciuri mecA* copy, sustaining our previous proposal that this element belongs to the genetic background of *S. sciuri*. Moreover, 46% of the strains also carried the MRSA *mecA* copy. Only these strains showed significant levels of resistance to beta-lactams. Strikingly, the majority of the strains carrying the additional MRSA *mecA* copy were obtained from healthy individuals in an antibiotic-free environment. Most of the 28 strains were resistant to penicillin, intermediately resistant to clindamycin, and susceptible to tetracycline, erythromycin, and gentamicin. Resistance to these last three antibiotics was found in some strains only. The findings reported in this work confirmed the role of *S. sciuri* in the evolution of the mechanism of resistance to methicillin in staphylococci and suggested that this species (like the pathogenic staphylococci) may accumulate resistance markers for several classes of antibiotics.

Staphylococcus sciuri was first described by Kloos and colleagues in 1976 (15) and is considered one of the most ancestral and dispersed staphylococcal species, with a wide range of habitats that includes the skin of several animals as well as environmental reservoirs, such as soil, sand, water, and furniture (14, 15, 16, 17). The impressive colonizing capacity of this species may result from its broad range of biochemical activities, which includes the ability to use inorganic nitrogen salts as the sole source of nitrogen. Traditionally described as a commensal species of rodents, marsupials, cetaceans, artiodactyls, and perissodactyls, *S. sciuri* has also been isolated from healthy and sick domestic and husbandry animals, including household cats (4, 12), domestic dogs (15), cattle, goats, poultry, sheep, horses, and pigs (3, 7, 8, 13, 27), and houseflies (9). Although *S. sciuri* is associated rarely with colonization or infection in humans (14), it has been occasionally isolated from human clinical samples (1, 3, 6, 10, 11, 17, 18, 21, 29, 31).

In an earlier report (3), we described a collection of 134 *S. sciuri* isolates, mainly of animal origin, all carrying a homologue of the *mecA* gene present in methicillin-resistant *Staphylococcus aureus* (MRSA) strains and other methicillin-resistant pathogenic staphylococci. The homology between the *mecA* sequences found in *S. sciuri* and MRSA (79.5% DNA sequence similarity and 87.7% amino acid sequence similarity) (32) and the ubiquitous presence of *mecA* sequences in the *S. sciuri* chromosome led to the proposal that *mecA* might be a native gene of *S. sciuri* and the ancestor of the *mecA* element carried by MRSA. In the same study and another study, we also described five *S. sciuri* isolates which carried, in addition to *S. sciuri mecA*, a second copy of *mecA*, identical to the one in MRSA (3, 33).

In the present work, we analyzed a new collection of 28 *S. sciuri* strains, all isolated from humans, including healthy and hospitalized individuals, with three major aims: first, to assay the genomic diversity of several *S. sciuri* isolates recovered from individuals sharing a common environment, in order to gather additional data on the main patterns of *S. sciuri* colonization and dissemination among humans; second, to assay the presence of both variants of the *mecA* gene among the isolates; and third, to search for any correlation between carriage or infection caused by strains with the MRSA *mecA* gene and antibiotic consumption.

MATERIALS AND METHODS

Bacterial strains. The *S. sciuri* strains characterized in this study are listed in Table 1 and were from two distinct sources: healthy human carriers (adults and children) and hospitalized patients.

The strains isolated from healthy carriers were sampled over 3 years in the context of two projects for the surveillance of antibiotic-resistant bacteria in the community (I. Santos Sanches, R. Sá-Leão, I. Bonfim, D. Oliveira, R. Mato, M. Aires de Sousa, A. Brito Avô, J. Saldanha, A. Pereira, G. A. Olim, and H. de Lencastre, Abstr. 20th Int. Congr. Chemother., abstr. 4317, p. 154, 1997; H. de Lencastre et al., unpublished data). The 23 *S. sciuri* strains included in the present study were isolated from draftees in one barrack of the Portuguese Air Force Base Aérea da Ota (strains SS-1 to SS-31) and from children attending day-care centers (strains SS-34 and SS-37) (Table 1).

The remaining *S. sciuri* strains were isolated within the scope of other projects for the surveillance of antibiotic-resistant bacteria among clinical isolates. Strain SS-38 was isolated in Hospital Casa de Saúde de Santa Marcelina (São Paulo, Brazil), and strains SS-39 to SS-42 were isolated in two different hospitals in Cape Verde (Hospital Agostinho Neto, Cidade da Praia, Cape Verde, and Hospital Baptista de Sousa, Mindelo, Cape Verde) (M. Aires de Sousa et al., unpublished data).

Among the strains recovered from draftees, most *S. sciuri* strains were isolated from the axillae (76%) and the remaining ones were isolated from the nares. Strains from children attending day-care centers were isolated from nasopharyngeal specimens. Among the five clinical isolates, four *S. sciuri* isolates were isolated from colonized sources and one (SS-38) was isolated from an infected source (Table 1).

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TABLE 1. *S. sciuri* isolates analyzed in this study

Source	Strain	Original name	Sampling date (mo/yr)	Sex	Age (yr)	Site	Origin	Ward
Healthy carriers	SS-3	r48a	4/96	Male	19	Axillae	Portuguese Air Force	
	SS-5	r125a	4/96	Male	19	Axillae	Portuguese Air Force	
	SS-6	r146n	4/96	Male	19	Nares	Portuguese Air Force	
	SS-10	r231n2	5/96	Male	19	Nares	Portuguese Air Force	
	SS-11	r339a	6/96	Male	19	Axillae	Portuguese Air Force	
	SS-13	r347a	6/96	Male	20	Axillae	Portuguese Air Force	
	SS-1	r353n	6/96	Male	19	Nares	Portuguese Air Force	
	SS-16	r359a	6/96	Male	21	Axillae	Portuguese Air Force	
	SS-17	r365n	6/96	Male	20	Nares	Portuguese Air Force	
	SS-18	r369a	6/96	Male	17	Axillae	Portuguese Air Force	
	SS-19	r369n	6/96	Male	17	Nares	Portuguese Air Force	
	SS-20	r390a	6/96	Male	20	Axillae	Portuguese Air Force	
	SS-23	r569a2	10/97	Male	18	Axillae	Portuguese Air Force	
	SS-24	r649a2	10/97	Male	17	Axillae	Portuguese Air Force	
	SS-25	r648a2	10/97	Male	17	Axillae	Portuguese Air Force	
	SS-26	r886a1	6/98	Male	18	Axillae	Portuguese Air Force	
	SS-27	r889a	6/98	Male	18	Axillae	Portuguese Air Force	
	SS-28	r897a	6/98	Male	19	Axillae	Portuguese Air Force	
	SS-29	r939a1	6/98	Male	18	Axillae	Portuguese Air Force	
	SS-30	r959a	6/98	Male	19	Axillae	Portuguese Air Force	
	SS-31	r969a	6/98	Male	20	Axillae	Portuguese Air Force	
	SS-34	S219	2/96	Female	6 mo	Nasopharynx	Portugal, day-care center 2	
	SS-37	S650	1/97	Male	2	Nasopharynx	Portugal, day-care center 1	
Clinical isolates	SS-38	BRA42	6/97	Female	65	Blood	Brazil, Casa de Saúde de Santa Marcelina	Medicine
	SS-39	CV3	4/97	Female	15	Sputum	Cape Verde, Hospital Agostinho Neto	Orthopedics
	SS-40	CV4	2/97	Male	12	Nose	Cape Verde, Hospital Agostinho Neto	Orthopedics
	SS-41	CV99	7/97	Male	77	Nose	Cape Verde, Hospital Baptista de Sousa	Medicine
	SS-42	CV134	3/97	Male	33	Nose	Cape Verde, Hospital Baptista de Sousa	Surgery

A questionnaire in which current and recent antibiotic use, antibiotic consumption habits, and recent hospital attendance were evaluated was given to all healthy participants (draftees and children). Since 1997, an additional question was introduced in the draftee questionnaire; it concerned frequent contact with animals.

Control strains were obtained from the culture collections of the Laboratory of Microbiology of the Rockefeller University and of the Molecular Genetics Unit of Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa. *S. aureus* strains RN2677 and COL were used as methicillin-susceptible *S. aureus* and MRSA controls, respectively, and *S. aureus* strain ATCC 25923 was used as a control for susceptibility testing. Additional controls included the type strains of the three *S. sciuri* subspecies, *S. sciuri* subsp. *sciuri* K1 (ATCC 29062^T), *S. sciuri* subsp. *rodentium* K9 (ATCC 70061^T), and *S. sciuri* subsp. *carnaticus* K128 (ATCC 70058^T), and an additional *S. sciuri* subsp. *rodentium* strain, K3 (3, 17). *Escherichia coli* strain MC1061-1 containing plasmid pMF13 with the *mecA* probe (20) was obtained from the same culture collections.

Media and growth conditions. All strains were aerobically grown at 37°C. *S. aureus* and *S. sciuri* strains were grown in tryptic soy broth or agar (Difco Laboratories, Detroit, Mich.). Luria-Bertani medium (26) supplemented with 50 μg of ampicillin (Sigma Chemical Company, St. Louis, Mo.) per ml was used to grow *E. coli* strain MC1061-1.

Species identification tests. Species identification tests included a catalase assay with 3% hydrogen peroxide to detect the presence of cytochrome oxidase; mannitol fermentation, by spreading overnight cultures with a sterile inoculation loop on mannitol salt agar (Difco) and incubating them at 37°C for 20 h; and coagulase production with the Bacto Coagulase Plasma test (Difco) according to the manufacturer's instructions. Detection of cytochrome *c* (modified oxidase test) was carried out with DrySlide Oxidase (BBL Microbiology Systems, Cockeysville, Md.) according to the manufacturer's instructions. Further biochemical profiles were determined with the ID32 STAPH system (bioMérieux Vitek Inc., Hazelwood, Mo.).

Antimicrobial susceptibility testing. Antibiotic susceptibilities were determined by the Kirby-Bauer technique with Muller-Hinton agar (Difco) according to National Committee for Clinical Laboratory Standards recommendations and definitions (22). The following antibiotic disks (BBL) were used: penicillin (10 μg), tetracycline (30 μg), erythromycin (15 μg), gentamicin (30 μg), clindamycin (12 μg), and novobiocin (5 μg). *S. aureus* ATCC 25923 and the type strains of the three *S. sciuri* subspecies were used as controls. The breakpoints used for novobiocin resistance were inhibition zones up to 12 mm for resistant strains and larger than 16 mm for susceptible strains (19).

Detection of beta-lactamase production. The beta-lactamase assay was carried out with DrySlide Nitrocefin (Difco) according to the manufacturer's instructions.

PAPs for oxacillin. Population analysis profiles (PAPs) were determined as recently described (24). Briefly, 10-μl drops of several dilutions (from 10^0 to 10^{-5} or 10^{-7}) of aerobically grown overnight cultures were spotted on the tops of Falcon Integrid square plates (100 by 15 mm) (BBL) containing tryptic soy agar with serial (twofold) dilutions of oxacillin (Sigma) at concentrations of 0 and 0.75 to 800 μg/ml. After inoculation, the plates were held vertically for a few seconds to allow the spread of the cultures across the surfaces of the plates. Colonies were counted after incubation for 48 h at 37°C. A graphic representation (PAP) was constructed by plotting the logarithm of colony counts against the concentration of oxacillin. The MIC was defined as the lowest concentration of antibiotic that inhibited the growth of 99.9% of cells.

Preparation of chromosomal DNAs for conventional and pulsed-field gel electrophoresis (PFGE). Chromosomal DNAs were prepared as previously described (5) with the following modifications for *S. sciuri* strains: EC buffer was supplemented with 0.5% Brij 58 (Sigma), and lysis took place for 5 h at 37°C.

Restriction digestion. Restriction digestion with *Bsp*106 (an isoschizomer of *Cla*I) and *Sma*I was performed according to manufacturer recommendations (Stratagene and New England Biolabs, respectively).

Conventional gel electrophoresis. Conventional gel electrophoresis was performed with 1% LE agarose (FMC BioProducts, Rockland, Maine) gels in Tris-acetate-EDTA (TAE) buffer (26) for 14 to 16 h at 1.5 V/cm.

PFGE. PFGE was carried out with a contour-clamped homogeneous electric field apparatus (CHEF-DRII; Bio-Rad, Hercules, Calif.) as previously described (25). Analysis of *Sma*I macrorestriction profiles was done by visual inspection, and PFGE patterns were assigned using the criteria proposed by Tenover and colleagues (30). Isolates with an identical PFGE pattern were included in the same type, designated by an uppercase letter. Isolates with PFGE types differing

by up to six fragments were assigned to subtypes, identified by uppercase letters followed by numerical codes.

Hybridization with the *mecA* probe. *Cla*I and *Sma*I DNA fragments in conventional and PFGE gels were transferred by vacuum blotting as previously described (5). The *mecA* probe used was the 1.196-kb *Xba*I-*Pst*I fragment from the *mecA* gene of the Australian MRSA strain ANS46 cloned in plasmid pTZ19 (20). For probe labeling and hybridization, an enhanced chemiluminescence nonradioactive labeling kit (RPN3040; Amersham Life Science, Arlington Heights, Ill.) was used according to the manufacturer's instructions.

Detection of the *S. aureus mecA* and *mecI* and *S. sciuri mecA* sequences. Detection of gene sequences was carried out by PCR amplification of chromosomal DNA with specific primers: for *S. sciuri mecA*, we used primers SAMECA358 (5′-ATCCATCAATATTGAACCA) and SAMECA1482 (5′-TATCTTCACCAACACC); for *S. aureus mecA*, we used primers SAMECA349 (5′-GTTAAAGAAGATGGTATG) and SAMECA1482; and for *S. aureus mecI*, we used primers MECI1 (5′-GTATGAAATATCATCTGCAG) and MECI2 (5′-AACAGAGGAAATATTCAACG). Amplifications were carried out with the Perkin-Elmer Cetus (Norwalk, Conn.) PCR reagent kit according to the manufacturer's instructions and with the following amplification protocol: 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C for 25 cycles and a final extension at 72°C for 4 min. Amplification products were resolved in 0.8% LE agarose (FMC BioProducts) gels in TAE.

RESULTS

Twenty-eight *S. sciuri* isolates recovered from 27 individuals (20 draftees, 2 children, and 5 hospital patients) were included in this study (Table 1).

Biochemical profile. All *S. sciuri* isolates showed a similar biochemical profile, as determined by the ID32 STAPH system. Variations were observed in the fermentation of lactose, ribose, *N*-acetylglucosamine, turanose, and arabinose and the production of alkaline phosphatase. Some isolates produced beta-glucuronidase. Two isolates (SS-5 and SS-24) could not be identified with this system because they failed to reduce nitrates. However, their similarity to the other isolates, namely, the remaining biochemical profile and PFGE type (see below), led us to classify them as *S. sciuri*. Moreover, these two isolates resembled another *S. sciuri* strain, designated K2 or BT22 and described earlier (3, 17), which was also reported as unable to reduce nitrate to nitrite (17). Two other strains (SS-11 and SS-20) had subpopulations detected in the oxacillin disk assay. The colonies grown outside and inside the halo had different behaviors toward ribose fermentation according to the ID32 STAPH system; the more resistant subpopulations failed to produce ribose. These subpopulations also grew slowly on blood agar plates, producing only small colonies.

Antibiotic susceptibility testing. As expected, all 28 *S. sciuri* isolates were resistant to novobiocin, which is a characteristic of this species. Twenty-one isolates shared a common antibiotype that included additional resistance to penicillin, intermediate resistance to clindamycin, and susceptibility to tetracycline, erythromycin, and gentamicin. Exceptions to this pattern were found in seven isolates and are detailed in Table 2. The MICs of oxacillin for 17 isolates were low (0.75 to 6 μg/ml). The majority of these isolates (13 of 17) had subpopulations resistant to up to 3 to 6 μg/ml. The MICs for 11 of the 28 isolates were higher (12 to 25 μg/ml), and there were subpopulations resistant to up to 800 μg of oxacillin per ml. The majority of the isolates (79%) produced beta-lactamase.

Genotypic analysis. (i) PFGE patterns. The 28 *S. sciuri* isolates could be assigned to 16 PFGE types (Table 2 and Fig. 1A); four of them had two or more different subtypes (Table 2 and Fig. 1A). The most common PFGE patterns were A and B (each with five isolates), C (four isolates), and D (two isolates). The remaining 12 PFGE patterns (corresponding to 43% of the isolates) were represented by single isolates only. Although PFGE patterns A, B, and C had more than six band differences, according to the interpretation proposed by Tenover and colleagues (30), we should emphasize that their similarity suggests that isolates with these PFGE patterns have probably evolved from a single strain, as can be observed by visual inspection of the macrorestriction profiles (Fig. 1A).

Strains with the same PFGE pattern were isolated from different draftees in more than one sampling period (1996 through 1998). For example, strains with PFGE pattern B were isolated in two months in 1996 (isolates SS-3, SS-17, and SS-19), in 1997 (SS-24), and in 1998 (SS-28). Also, strains with PFGE pattern A were recovered in 1996 and 1998.

One individual (a draftee) carried two different *S. sciuri* strains, as determined by their distinct PFGE profiles. One was isolated from the axillae (SS-18), and the other was isolated from the nares (SS-19).

(ii) Localization of the *mecA* sequences in PFGE profiles. Hybridization of *Sma*I chromosomal digests with a DNA probe internal to the *mecA* gene of an MRSA strain showed that 13 isolates hybridized with this probe in two *Sma*I bands (Table 2 and Fig. 1B). The molecular size of the hybridizing *Sma*I bands ranged from 140 to 490 kb. Twelve of the 15 isolates with a single *Sma*I-*mecA* hybridization band carried the *mecA* copy in a high-molecular-size (374 to 520 kb) *Sma*I fragment. The other three isolates (SS-34, SS-37, and SS-41) hybridized with the *mecA* probe in smaller *Sma*I bands, ranging from 138 to 164 kb.

(iii) Analysis of the *mecA* copies. All isolates with two *Sma*I-*mecA* hybridization bands had three *Cla*I-*mecA* hybridization bands, corresponding to the two expected bands of MRSA *mecA* and the single band of *S. sciuri mecA*; this result is in accordance with our previous observation that unlike *mecA* of MRSA strains, *S. sciuri mecA* has no restriction sequence for *Cla*I (3, 32). On the other hand, isolates with only one *Sma*I-*mecA* hybridization band had a single *Cla*I-*mecA* hybridization band (Fig. 2), suggesting that *mecA* carried by these isolates is the *S. sciuri* native copy.

This interpretation was confirmed by PCR amplification of the two types of *mecA* with primers specific for the *S. aureus* or the *S. sciuri mecA* copies. While all *S. sciuri* isolates produced a 1.12-kb amplification fragment with the primers specific for *S. sciuri mecA*, only isolates showing extra hybridization bands in the *Sma*I-*mecA* and *Cla*I-*mecA* gels produced a second amplification band with the primers specific for MRSA *mecA*. Moreover, only these isolates produced amplification bands with primers specific for the *mecI* gene (Table 2).

Expression of resistance to oxacillin. For all *S. sciuri* isolates with both copies of *mecA*, the MICs of oxacillin ranged from 3 to 25 μg/ml; the MICs for the majority (77%) were 12 or 25 μg/ml, with subpopulations able to grow in the presence of up to 800 μg of antibiotic per ml. On the other hand, for all but two isolates with a single copy of *mecA*, the MICs were lower (0.75 to 3 μg of oxacillin per ml), and no subpopulations were detected at antibiotic concentrations of higher than 6 μg/ml. The two exceptions found were strains SS-37 and SS-41. Although both showed single *Sma*I-*mecA* and *Cla*I-*mecA* hybridization bands, the MIC for strain SS-37 was 25 μg/ml, with subpopulations able to grow in the presence of up to 800 μg/ml, while for strain SS-41, the MIC was low (3 μg/ml), with subpopulations able to grow in the presence of up to 50 μg/ml (Fig. 3).

DISCUSSION

In this work, we characterized a group of 28 *S. sciuri* isolates from human origin, mostly from colonization sources. Twenty-three out of the 28 isolates described in this study were found among a collection of 252 staphylococcal isolates recovered from healthy carriers—selected on the basis of their resistance

TABLE 2. Phenotypic and genotypic characterization of the *S. sciuri* isolates

Source	Strain	ID32 STAPH result[a]	Sampling date (mo/yr)	PFGE profile	*Sma*I-*mecA* band (kb)	*Cla*I-*mecA* band (kb)	PCR amplification[b]			Oxacillin (μg/ml)		Strains resistant (r) or intermediate (i) to[c]:	β-Lactamase production[d]
							S. sciuri mecA	MRSA		MIC	Concn up to which strains were resistant		
								mecA	*mecI*				
Draftees	SS-1	++	6/96	A1	490, 225	11.7, 2.2, 1.5	+	+	+	12	800	NOV^r, PEN^r, CLI^i	+
	SS-30	++	6/98	A1	490, 225	11.7, 2.2, 1.5	+	+	+	12–25	800	NOV^r, PEN^r, CLI^i	+
	SS-6	++	4/96	A2	490, 225	11.7, 2.2, 1.5				25	800	NOV^r, PEN^r, CLI^i	+
	SS-11	++	6/96	A3	490, 225	11.7, 2.2, 1.5				25	800	NOV^r, PEN^r, CLI^i	+
	SS-31	++	6/98	A4	490, 225	11.7, 2.2, 1.5				25	800	NOV^r, PEN^r, CLI^i	+
	SS-3	++/+	4/96	B1	490	11.7	+	−	−	0.75	3	NOV^r, PEN^r, CLI^i	+
	SS-17	+/++	6/96	B1	490	11.7				0.75	3	NOV^r, PEN^r, CLI^i	+
	SS-19	+/++	6/96	B1	490	11.7				0.75	3	NOV^r, PEN^r, CLI^i	+
	SS-28	++/+++	6/98	B1	490	11.7				0.75	3	NOV^r, PEN^r, CLI^i	+
	SS-24	None; Nit$^-$	10/97	B2	490	11.7				1.5	1.5	NOV^r, PEN^r, CLI^i	+
	SS-10	++	5/96	C1	490, 195	11.7, 2.2, 1.4	+	+	+	12	800	NOV^r, PEN^r, CLI^i	+
	SS-13	++	6/96	C1	490, 195	11.7, 2.2, 1.4				12	800	NOV^r, PEN^r, CLI^i	+
	SS-26	++/+++	6/98	C1	490, 195	11.7, 2.2, 1.4				12	800	NOV^r, PEN^r, CLI^i	+
	SS-20	++	6/96	C2	490, 195	11.7, 2.2, 1.4				12	800	NOV^r, PEN^r, CLI^i	+
	SS-5	None; Nit$^-$	4/96	E	485	11.5	+	−	−	0.75	3	NOV^r, PEN^r, CLI^i	+
	SS-16	++	6/96	F	520	11.5	+	−	−	0.75	1.5	NOV^r, PEN^r, CLI^i	+
	SS-18	+++/++	6/96	G	485	11.5	+	−	−	1.5	3	NOV^r, PEN^r, CLI^i	+
	SS-23	++	10/97	H	490	11.5				0.75	1.5	NOV^r, PEN^r, CLI^i	+
	SS-25	+++	10/97	I	485, 255	11.5, 5.2, 2.2	+	+	+	6	400	NOV^r, PEN^r, CLI^i	−
	SS-27	++/+++	6/98	J	485	11.5				1.5	1.5	NOV^r, PEN^r, CLI^i	+
	SS-29	+++	6/98	K	485, 175	14.8, 6.7, 2.2	+	+	+	25	800	NOV^r, PEN^r, CLI^i, ERY^r	+
Day-care center children	SS-34	+	2/96	L	140	11.5	+	−	−	1.5	1.5	NOV^r, CLI^r	+
	SS-37	+++	1/97	M	150	8.2	+	−	−	25	800	NOV^i, PEN^r, GEN^r	−
Clinical isolates	SS-38	+++	6/97	N	480	11.5	+	−	−	3	6	NOV^i, TET^r	−
	SS-39	+	4/97	D1	185, 150	5.9, 4.4, 2.2	+	+	+	3	800	NOV^r, PEN^r, CLI^i, GEN^r	−
	SS-40	+	2/97	D2	185, 160	6.9, 5.8, 2.2				3	800	NOV^i, PEN^r, CLI^i, GEN^r, ERY^r	−
	SS-41	+++	7/97	O	165	13.5	+	−	−	3	50	NOV^r, PEN^r	−
	SS-42	+++	3/97	P	375	11.5	+	−	−	1.5	1.5	NOV^i, PEN^r, CLI^i	+
Control strains													
S. sciuri	ATCC 29062^T (K1)				>500	15.0	+	−	−	3	6	NOV^r, CLI^r	−
	ATCC 0061^T (K9)				160	13.5	+	−	−	3	6	NOV^r, CLI^r	
	ATCC 00058^T (K128)				145	12.3	+	−	−	1.5	3	NOV^r, PEN^r, ERY^r, TET^i	
	K3				175, 150	13.5, 5.4, 2.2	+	+	+	6	800		+
S. aureus	COL				200	12.3, 2.1	−	+	−	800	800		−
	RN2677						−	−	−	0.75			−

[a] +++, ++, and +, classified as *S. sciuri* with an excellent, very good, and good identification, respectively (see Materials and Methods).
[b] +, amplification; −, no amplification.
[c] Panel of antibiotics tested: penicillin (PEN), clindamycin (CLI), tetracycline (TET), erythromycin (ERY), gentamicin (GEN), and novobiocin (NOV).
[d] +, production; −, no production.

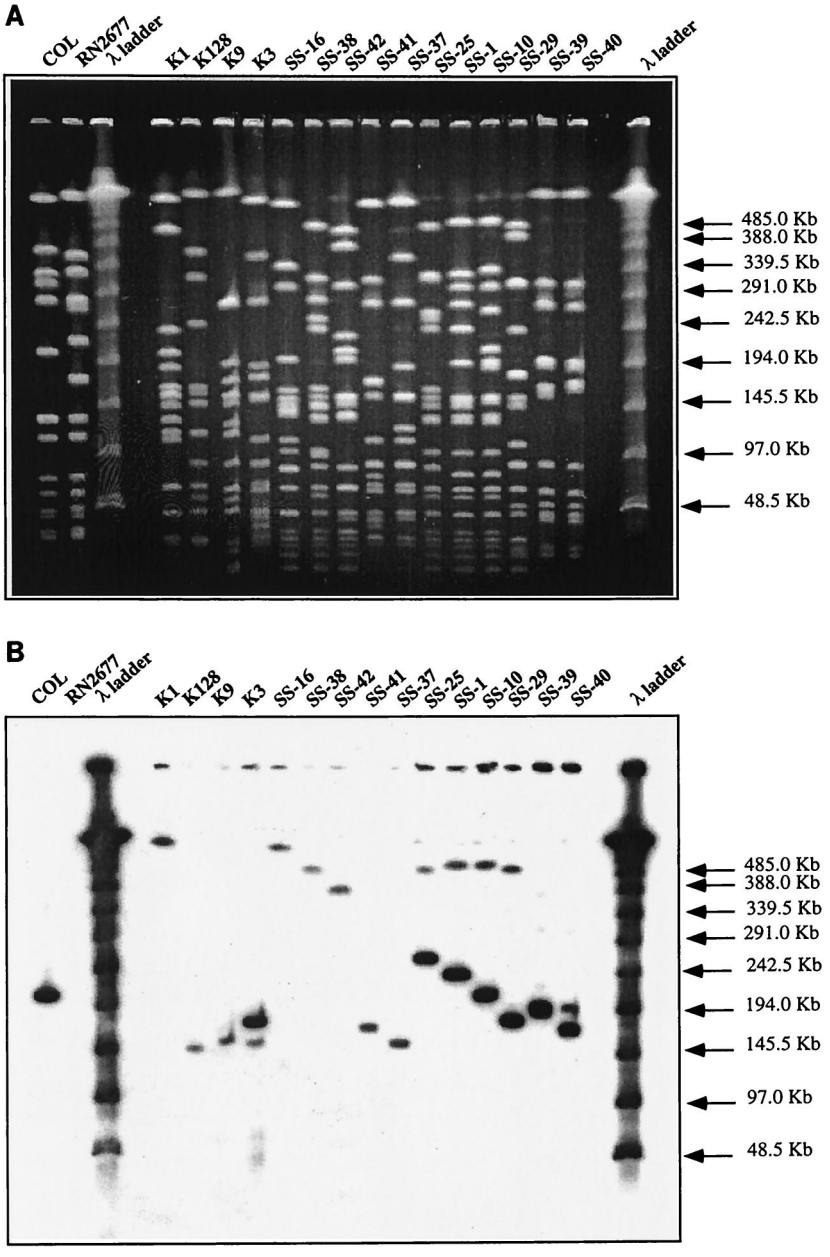

FIG. 1. (A) PFGE patterns of *S. sciuri* strains after *Sma*I digestion. (B) Hybridization of *S. sciuri Sma*I digests with an *S. aureus mecA* probe.

to oxacillin in the disk diffusion method (22)—and characterized in our laboratory (H. de Lencastre et al., unpublished data). Although the percentage of *S. sciuri* isolation relative to that of other staphylococci may vary with an increase in the number of isolates analyzed (or the use of new resistance breakpoints [23]), we speculate that *S. sciuri* may be more relevant as a human colonizer species than has been considered.

Some of the *S. sciuri* isolates recovered from different individuals (draftees) over a 3-year period were clonally related, as determined by PFGE analysis. The clonal dissemination of these strains within individuals in this barrack and their persistence in this environment over a 3-year period may be explained by the high survival skills of this bacterium, which allow its residence in an environmental niche that would represent a source for the continuous contamination of individuals. It has been claimed that the isolation of *S. sciuri* in humans is a result of close contact with animals. At the time of sampling, the population studied had no permanent contact with animals, and among individuals who did have sporadic contact, the majority carried strains with PFGE patterns already found in previous years. These results sustain our previous hypothesis that these *S. sciuri* strains were acquired in the barrack environment rather than from animals.

Another interesting result was the isolation of *S. sciuri* strains among clinical isolates, particularly from one infection source (blood). Two other reports also documented the isolation of this species from blood samples (11, 31), and other authors mentioned the isolation of *S. sciuri* strains from other clinical sources, such as infected wounds of hospital patients

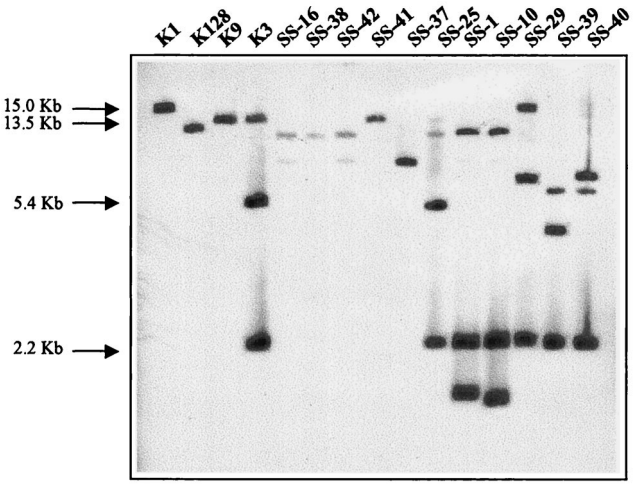

FIG. 2. Polymorphisms in the *S. sciuri mecA* vicinity after hybridization of *Cla*I digests with an *S. aureus mecA* probe.

(18, 31) and umbilici of infants and the teats of their mothers (17). Furthermore, several strains of *S. sciuri* were reported to be adherence positive (10) or to produce slime (31), which is considered a potential factor for both colonization and virulence. Nevertheless, the paucity of reports of infections caused directly by *S. sciuri* indicates that it is probably a rare and opportunistic pathogen in humans. In fact, besides the study of Hedin and Widerström (11) that clearly identified *S. sciuri* as the bacterium responsible for an endocarditis case, all the other reports of clinical *S. sciuri* did not prove unequivocally that this was the agent responsible for the infections reported. Our own observation that *S. sciuri* may colonize humans more frequently than previously thought may explain the recovery of this bacterium from clinical samples.

Although the clinical significance of *S. sciuri* may remain controversial, the capacity of this species to carry resistance determinants is well established. It is known that *S. sciuri* strains may carry plasmids with antibiotic resistance markers (28), and some clinical isolates were found to be multiresistant (18, 31). Kawano and colleagues (13) described the isolation from healthy chickens of *S. sciuri* strains resistant to several classes of antibiotics; many of the strains studied by Kloos et al. (17), mostly isolated from wild animals, showed resistance patterns comparable to the ones described in this work, with the exception that most of the strains studied by those authors were susceptible to clindamycin. In our work, no obvious differences were seen among the resistance patterns of the strains isolated from healthy carriers or hospital patients, although the clinical strains showed some additional antibiotic markers.

All *S. sciuri* strains characterized in this study carried the *S. sciuri mecA* copy. This result confirms our earlier findings (3) and further supports the hypothesis that this is a native element of the *S. sciuri* chromosome. Furthermore, a high percentage of isolates (46%) also carried the MRSA *mecA* copy. This finding is even more striking if we consider that most isolates carrying MRSA *mecA* (11 out of 13) were isolated from healthy individuals. Of these, only one person (carrier of strain SS-26) had taken antibiotics, namely, amoxicillin-clavulanic acid, during the month previous to sampling. Therefore, no correlation was found between carriage of *S. sciuri* with MRSA *mecA* and antibiotic consumption. Similarly, it was not possible to find any correlation between the presence of these strains and attendance at hospitals, since only three individuals had been in a hospital recently and, of these, only one carried a strain (SS-20) with the MRSA *mecA* gene.

MRSA *mecA* was found in a heterogeneous chromosomal background, since five different strains carried this element. Four out of these five strains were isolated from healthy individuals. This is an interesting result, because it illustrates the in vivo dissemination of MRSA *mecA* in an antibiotic-free environment. In addition, all *S. sciuri* isolates carrying MRSA *mecA* also carried the *mecI* element. The simultaneous presence of both sequences strongly supports the hypothesis that the MRSA-like elements were recently acquired from an exogenous donor, probably a pathogenic species of staphylococci, followed by their spread within different *S. sciuri* strains. We had previously reported the presence of MRSA *mecA* in *S. sciuri* isolated from human samples (3); however, in the previous study, all the *S. sciuri* isolates carrying MRSA *mecA* were clonally related and were isolated from a single hospital ward, suggesting that *mecA* transfer from a pathogenic, MRSA strain had occurred once, followed by clonal dissemination of the *S. sciuri* strain carrying the newly acquired MRSA *mecA* gene. The results presented in this work seem to illustrate this event as well as the transfer of MRSA *mecA* among different *S. sciuri* strains.

In this same previous study, it was reported that *S. sciuri mecA* was not able to confer significant resistance to beta-lactam antibiotics (3). This observation was confirmed in the present study. Comparison between the profiles of resistance toward oxacillin of the *S. sciuri* strains with one or two *mecA* copies clearly indicated that only strains with the MRSA *mecA* copy are able to grow in the presence of this antibiotic. However, two exceptions were found, strains SS-37 and SS-41. Although both strains carried only *S. sciuri mecA*, their PAPs resembled those of strains with both *mecA* copies. Furthermore, both strains were resistant to penicillin but failed to produce beta-lactamase, indicating that the *mecA* copy present in their chromosomes conferred resistance to beta-lactams. This *mecA* copy could be amplified with primers specific for *S. sciuri mecA* but not with primers specific for MRSA *mecA*, thus excluding the hypothesis of the presence of a single MRSA *mecA* copy. Therefore, it seems that the *mecA* gene found in these strains is able to confer the same level and type of resistance as the copy carried by MRSA, a finding to be further analyzed in future work. Further studies should also focus on

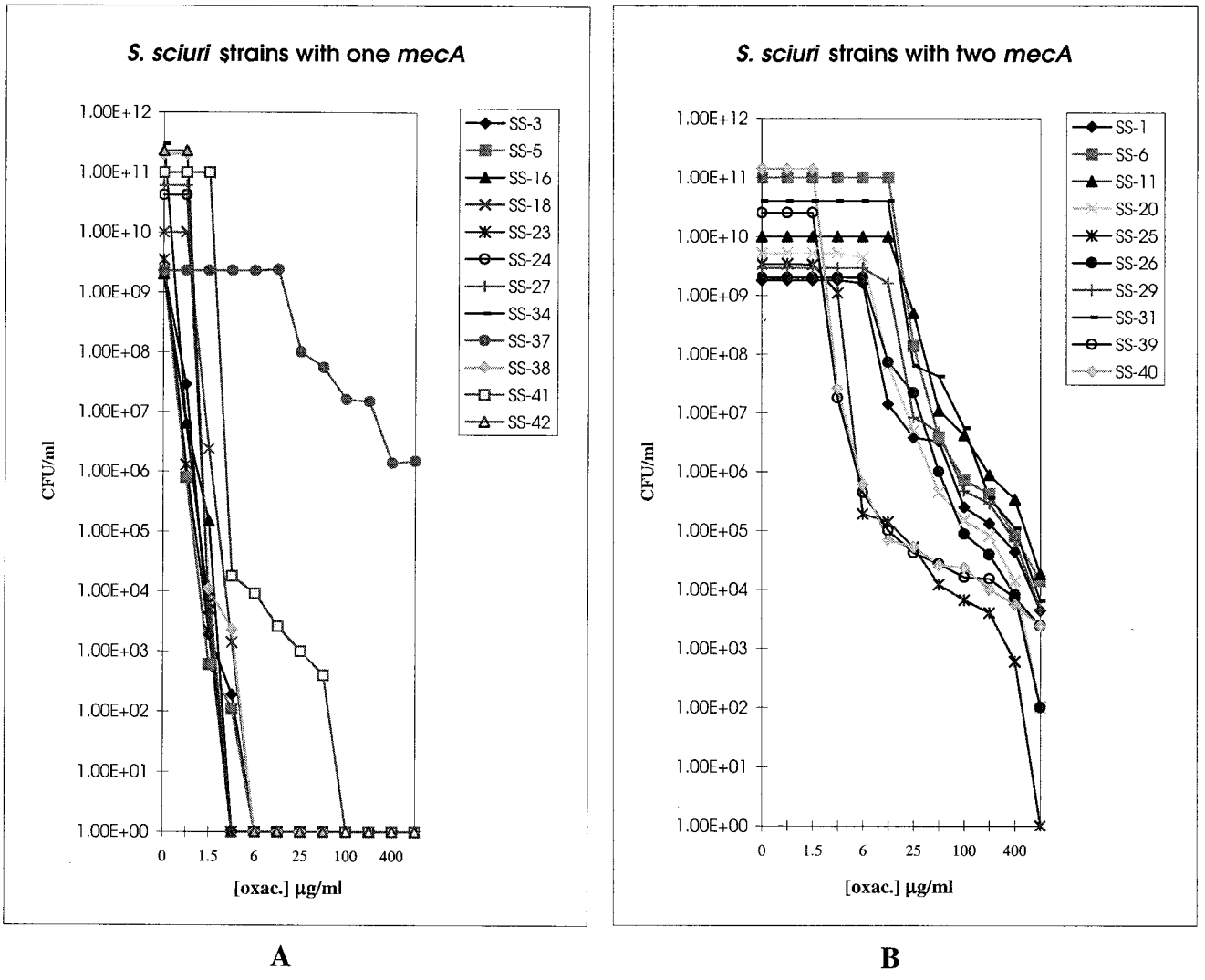

FIG. 3. PAPs of analyzed *S. sciuri* strains with only one *mecA* copy (A) or two *mecA* copies (B). oxac., oxacillin.

the presence of *S. sciuri* strains in human samples and their relationship to contacts with animal or environmental contamination, in order to establish the real risk factors and impact of human colonization by *S. sciuri* as well as to address the pathogenicity of this species.

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