Ribotype Delineation and Description of *Staphylococcus sciuri* Subspecies and Their Potential as Reservoirs of Methicillin Resistance and Staphylolytic Enzyme Genes

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Three subspecies of *Staphylococcus sciuri*, *S. sciuri* subsp. *sciuri* Kloos, Schleifer, and Smith 1976, 23^{AL} emend. Kloos et al. 1996, *S. sciuri* subsp. *carnaticus* subsp. nov., and *S. sciuri* subsp. *rodentium* subsp. nov., are described on the basis of their ribotype patterns, DNA-DNA liquid hybridization data, and phenotypic characteristics. Normalized ribotyping subdivided the *S. sciuri* patterns into three blocks of patterns, each corresponding to a subspecies. Each subspecies formed a separate, well-defined DNA similarity group when DNA-DNA hybridizations were conducted under stringent (70°C) reassociation conditions. *S. sciuri* subsp. *sciuri* could be distinguished from the other subspecies on the basis of its ability to produce acid from D-cellobiose, alkaline phosphatase activity, and inability to produce either clumping factor or protein A. *S. sciuri* subsp. *carnaticus* could be distinguished by its ability to produce acid aerobically from D-xylose and maltose, inability to produce acid from D-melezitose, and smaller colony size on P agar. *S. sciuri* subsp. *rodentium* could be distinguished by its positive reaction in the latex agglutination test for clumping factor and/or protein A and generally higher frequencies and levels of oxacillin and methicillin resistance. All 40 strains of *S. sciuri* tested (including representatives of all three subspecies) hybridized with the *mecA* gene probe. All strains of *S. sciuri* subsp. *sciuri*, 79% of the strains of *S. sciuri* subsp. *carnaticus* and 89% of the strains of *S. sciuri* subsp. *rodentium* exhibited extracellular, staphylolytic enzyme activity. This activity was associated with an enzyme(s) that immunoblotted with a lysostaphin-specific monoclonal antibody; however, only three strains hybridized with a lysostaphin (*end*) gene probe. The type strain of *S. sciuri* subsp. *carnaticus* is DD 791 (= ATCC 700058), and the type strain of *S. sciuri* subsp. *rodentium* is DD 4761 (= ATCC 700061).

Staphylococcus sciuri is generally considered one of the most primitive *Staphylococcus* species; it is widely distributed in nature, is capable of growth on inorganic nitrogen salts as the sole source of nitrogen, and exhibits a wide range of biochemical activities (31–33). In the past 20 years since its original description (33), numerous laboratories have reported frequent isolation of this species from foods, farm animals, rodents, marsupials, marine mammals, and birds and occasional isolation from humans and their pets (1, 2, 17, 28, 31, 35, 48, 52, 57, 58). Most resident populations of *S. sciuri* are members of the normal cutaneous microflora of lower mammals. This species is rarely associated with infections. A preliminary investigation of a large collection of *S. sciuri* strains showed that a ribotyping method based on an analysis of genomic *EcoRI* fragments containing portions of the rRNA operons could be used to distinguish two major subgroups and additional strain variation within the species (10).

In this study, we describe three subpopulations of *S. sciuri* which can be distinguished on the basis of their ribotype patterns, DNA-DNA liquid hybridization data, and phenotypic characteristics. Compared to the ribotypes representing the subpopulation of *S. sciuri* subsp. *sciuri*, the ribotypes representing a second subpopulation appeared to have one region ad-

jacent to an rRNA operon replaced in its entirety, while the ribotypes representing a third subpopulation appeared to have two regions adjacent to different rRNA operons replaced in their entirety. Each of these subpopulations has been given subspecies status. In this report, we include an amended description of *S. sciuri* subsp. *sciuri* (Kloos, Schleifer, and Smith 1976), describe two additional subspecies, *S. sciuri* subsp. *carnaticus* and *S. sciuri* subsp. *rodentium*, and designate the type strains of these taxa. The nature of two surprising phenotypic properties, common methicillin resistance and staphylolytic activity, were explored in some detail, because these properties indicate that *S. sciuri* is a natural reservoir of important methicillin resistance and staphylolytic enzyme genes and their products that are novel to certain other *Staphylococcus* species (5, 21, 39, 43, 53, 54).

MATERIALS AND METHODS

Bacterial strains. The designations and sources of the strains used to describe the *S. sciuri* subspecies are listed in Table 1. Unless indicated otherwise, all strains were isolated from the skin of mammalian hosts. Strains from the neonatal ward of the Department of Pediatrics, Maputo Central Hospital, Maputo, Mozambique, were isolated from the umbilici of infants, the teats of mothers, soaps, and the surfaces of incubator entrances.

The methicillin-resistant *Staphylococcus aureus* strains used as positive controls for the presence of the *mecA* gene were COL and N315. Strain RN2677, a methicillin-susceptible *S. aureus* strain, was used as a negative control. These strains were obtained from the Rockefeller University culture collection. The *Escherichia coli* strain containing the plasmid with the *S. aureus mecA* probe has been described previously (15, 38). Lysostaphin-producing *Staphylococcus simu-*

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TABLE 1. *S. sciuri* strains and their sources

Subspecies	Strain designations			Source
	DuPont	Kloos	ATCC ^a	
<i>S. sciuri</i> subsp. <i>sciuri</i>	DD 4277 ^T	SC 116 ^T	ATCC 29062 ^T	Eastern gray squirrel
	DD 4285	SC 255		Eastern gray squirrel
	DD 4287	SC 114		Eastern gray squirrel
	DD 4276	GV 252	ATCC 29061	Southern flying squirrel
	DD 4289	GV 212		Southern flying squirrel
	DD 5277	MM1b5		Eastern harvest mouse
	DD 5272	PC2D5		California mouse
	DD 5285	MO2-1		Prairie vole
	DD 4274	DM 128	ATCC 29059	Opossum
	DD 4278	DM 71		Opossum
	DD 4279	DM 81		Opossum
	DD 4281	DM 93		Opossum
	DD 5286	MR1P2		Red kangaroo
	DD 4747	BT 21		Beef tongue
	DD 4537	BT 22		Beef tongue
	DD 4526	BL 12		Beef lips
	DD 5262	CA2L1		Jersey cattle calf
	DD 5256	HE1L13		Jersey cattle heifer
	DD 5748	H2L28		Morgan horse
	DD 4731	H2F14		Morgan horse
	DD 4520	H4L2		Arabian horse
	DD 6059	TT12b3		Bottle-nosed dolphin
	DD 6044	TT4m2		Bottle-nosed dolphin
	DD 6069	GMWg1		Pilot whale
	DD 4292	CF 3		Domestic dog
	DD 4295	SS 226		Squirrel monkey
<i>S. sciuri</i> subsp. <i>carnaticus</i>	DD 791 ^T		ATCC 700058 ^T	Veal leg, sliced
	DD 792			Veal leg, sliced
	DD 4750	BL 2		Beef lips
	DD 5666	CA1F6		Jersey cattle calf
	DD 5268	CA1F14		Jersey cattle calf
	DD 5238	CA1L9		Jersey cattle calf
	DD 5254	HE1L18	ATCC 700060	Jersey cattle heifer
	DD 5255	HE1L19		Jersey cattle heifer
	DD 5248	HE1F6		Jersey cattle heifer
	DD 5251	HE2F4		Jersey cattle heifer
	DD 4530	C3F23		Holstein cattle cow
	DD 5234	B3L6		Aberdeen Angus bull
	DD 5284	MP1v1		Acouchy (Cavy)
	DD 6081	TT13b1	ATCC 700059	Bottle-nosed dolphin
<i>S. sciuri</i> subsp. <i>rodentium</i>	DD 4761 ^T	R1-33 ^T	ATCC 700061 ^T	Norway rat
	DD 4535	R1-12	ATCC 700063	Norway rat
	DD 4762	R1-27		Norway rat
	DD 4773	R1-39		Norway rat
	DD 4780	R3-133		Norway rat
	DD 4776	R4-151		Norway rat
	DD 4765	R4-154		Norway rat
	DD 4768	CR5-5		Cotton rat
	DD 4778	CR5-249		Cotton rat
	DD 5283	SVv1		European red squirrel
	DD 5282	SVv6		European red squirrel
	DD 6077	GMWg14	ATCC 700062	Pilot whale
	DD 6272	MM 2		Neonatal ward (Maputo)
	DD 6273	MM 3		Neonatal ward (Maputo)
	DD 6276	MM 8		Neonatal ward (Maputo)
	DD 6278	MM 11		Neonatal ward (Maputo)
	DD 6281	MM 16		Neonatal ward (Maputo)
	DD 6282	MM 17		Neonatal ward (Maputo)

^a ATCC, American Type Culture Collection, Rockville, Md.

lans biovar *staphylolyticus* strain NRRL B-2628 (53) was used as a positive control for staphylolytic activity, reactivity with a lysostaphin-specific monoclonal antibody (MAb), and hybridization with a lysostaphin gene (*end*) probe. The non-lysostaphin-producing and lysis assay organism *S. aureus* FDA 209P (= ATCC 6538P) was used as a negative control.

Phenotypic characterization. The following characteristics were determined as described previously (29, 30, 33, 59): Gram-stained cell morphology and cell arrangement, colony morphology and pigmentation, motility, anaerobic growth in thioglycolate semisolid medium, catalase activity, acetylmethylcarbinol (acetoin) production, nitrate reduction, oxidase activity, pyrrolidonyl arylamidase

activity, esculin hydrolysis, thermonuclease activity, ornithine decarboxylase activity, urease activity, staphylocoagulase activity, hemolysis of sheep, bovine, and horse blood, and carbohydrate reactions. The presence of clumping factor and/or protein A was tested by using a Staph Latex kit (Remel, Lenexa, Kans.) and Staph-A-Lex (Trinity Laboratories, Inc., Raleigh, N.C.). Antibiotic susceptibilities were determined by disk diffusion tests involving incubation of cultures on tryptic soy agar (TSA) (Difco Laboratories, Detroit, Mich.), Mueller-Hinton agar, and Mueller-Hinton agar containing 2% sodium chloride at 35°C for 24 h in the presence of various antibiotic disks, followed by measurement of diameters of zones of inhibition (halos) (60). All three of the media used in these tests produced similar results if they were freshly prepared and stored at 4°C for no longer than 1 week prior to use. Colony size, pigmentation, luster, profile, and consistency were determined on plates containing freshly prepared P agar (32), TSA, and TSA supplemented with 5% sheep blood following point inoculation, incubation for 72 h at 35°C, and storage at room temperature for an additional 2 days (29, 30). Alkaline phosphatase, urease, β -galactosidase, β -glucosidase, and β -glucuronidase activities and arginine utilization were tested with the API STAPH-IDENT system (bioMérieux Vitek, Hazelwood, Mo.). Additional biochemical profile data were obtained by using the STAPH Trac and ID32 STAPH systems (bioMérieux Vitek).

The extracellular staphylolytic enzyme activity of selected strains was evaluated by visualizing zones of bacteriolysis following growth on a modified lysostaphin production medium (45) that contained heat-killed cells of *S. aureus* ATCC 6538P, as described previously (46), and by renaturing activity gel analysis (41).

Normalized ribotyping. Discrete and reproducible ribotype descriptions of strains resulted from a single method described previously in detail (11, 24, 59). Briefly, strains were lysed with staphylolytic enzymes and sodium dodecyl sulfate (SDS). The solution was extracted with phenol and chloroform, and the DNA was precipitated with ethanol. The DNA was digested with *Eco*RI. The resulting fragments were separated by performing agarose gel electrophoresis in a minigel apparatus. The size-separated fragments were immobilized on a membrane by electrophoretic transfer, denatured, and probed. The *mb* ribosomal operon from *E. coli* (9), labeled by sulfonation, was used as a probe. Immobilized fragments selected by hybridization were detected with an antisulfonated DNA MAb conjugated to alkaline phosphatase and a chemiluminescent substrate. The image consisting of lanes of bands on the membrane was captured electronically and recorded. Computerized procedures were used to extract the strain data from each lane and to digitally process the bands to provide a normalized and reproducible characterization. Each strain was then represented in a database by a set of fragment bands. Sets from independently isolated strains that were indistinguishable within the experimental error were averaged, and the average was stored as a ribotype pattern type.

Genomic DNA-DNA hybridization. Unlabeled and [*methyl*-³H]thymidine-labeled total DNAs were isolated and purified by using the procedures of Brenner and coworkers (8), as modified by Kloos and Wolfshohl (34) and optimized for use with *S. sciuri* and other staphylococcal species that are somewhat difficult to lyse. Cultures were grown in tryptic soy broth (Difco) supplemented with glycine (10 to 15 g/liter) to produce lysis-susceptible cells for DNA isolation. The lysing buffer contained the following ingredients: glucose, 0.9 g; Tris, 0.39 g; EDTA (anhydrous), 0.29 g; chicken egg white lysozyme (Sigma Chemical Co., St. Louis, Mo.), 80 mg; Ambicin L (recombinant lysostaphin) (Aplin & Barrett Ltd., Clarks Mill, Trowbridge, Wilts, United Kingdom), 10 mg; and deionized H₂O, 100 ml. DNA-DNA reassociation reactions were performed under stringent (70°C) conditions to best identify subspecies populations, although several additional reactions were performed under optimal (55°C) conditions in order to make comparisons at the species level (27, 28). At the termination of reactions, double-stranded (hybridized) DNA was separated from unreacted, single-stranded DNA on hydroxyapatite by the batch procedure described by Brenner and coworkers (8). The relative binding (or DNA relatedness) was determined by normalizing the amount of DNA bound to hydroxyapatite in heterologous reactions to the amount bound in the homologous reaction, in which the labeled and unlabeled DNAs were from the same organism.

Cell wall analysis. Cell walls purified with 4% SDS by the procedures of Glauner et al. (20) were used to determine peptidoglycan composition and teichoic acid composition. Amino acid and teichoic acid analyses were performed as described previously (4, 26, 59). The levels of amino acids and amino sugars were determined by using a model LC6001 amino acid analyzer (Biotronik, Maintal, Germany). The peptidoglycan type was determined on the basis of the molar ratios of glutamic acid to L-lysine, to L-serine, to L-alanine, and to glycine (50). The sugar constituents of teichoic acids were determined by gas-liquid chromatography by using a Packard model GC 438 gas chromatograph equipped with a flame ionization detector (Packard Instrument Co., Meriden, Conn.) and a column (2 by 1,000 mm) filled with 3% SP 2340 on 100/200 Supelcoport (Supelco, Bellefonte, Pa.), and glycerol was detected enzymatically by the method of Bergmeyer (7). Phosphate levels were determined by the method of Ames (3).

DNA base composition. The guanine-plus-cytosine (G+C) content of DNA was determined by the thermal denaturation method of Marmur and Doty (37) by using a Guilford Response spectrophotometer with Response II thermal programming (Guilford Systems, Ciba Corning Diagnostics Corp., Oberlin, Ohio).

Dot blot hybridization with the *mecA* gene probe. Dot blot hybridization was performed as described previously (16, 49), with the following modifications. Overnight cultures (0.4 ml) were centrifuged and washed with 1 ml of Tris-EDTA buffer (TE). Cell walls were digested by adding 6 μ l of chicken egg white lysozyme (10 mg/ml in TE [pH 8.0]; Sigma), 15 μ l of lysostaphin (10 mg/ml in TE [pH 8.0]; Sigma), and 100 μ l of TE (pH 8.0) and incubating the preparation at 37°C for 1 h. The resulting protoplasts were lysed with 10 μ l of 10% SDS and 2 μ l of proteinase K (10 mg/ml; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) at 37°C for 1 h. The lysate was treated with phenol-chloroform-isoamyl alcohol, and the aqueous phase containing DNA was separated by centrifugation and denatured by adding 150 μ l of 3 M NaOH; this was followed by incubation at room temperature for 15 min. The samples were then chilled at 0°C, and the pH was neutralized by adding 150 μ l of 2 M sodium acetate. After centrifugation at 4°C, the supernatant fluids were transferred to prechilled tubes. A 25- μ l aliquot from each sample was loaded into a dot blot apparatus (Bio-Rad Laboratories, Hercules, Calif.). The DNA probe containing the *mecA* gene (38) was labeled with ³²P by using the Megaprime labeling system (catalog no. RPN1606; Amersham International plc, Little Chalfont, Bucks, United Kingdom) according to the manufacturer's instructions.

Quantitative analysis of staphylolytic enzyme production and lysostaphin susceptibility. A quantitative analysis of staphylolytic enzyme production (activity) in culture supernatants was performed by a standard tube assay for lysostaphin activity in which the lysis of a suspension of cells of *S. aureus* FDA 209P (optical density at 620 nm, 0.250) is monitored spectrophotometrically (44); 1 U of activity was defined as the amount of enzyme that caused a 50% reduction in turbidity in 10 min at 37°C. Quantitative determinations of lysostaphin susceptibility were determined spectrophotometrically as described previously (14) by using a final lysostaphin concentration of 1 U/ml.

Immunological analysis of staphylolytic enzymes. Culture supernatants were tested for reactivity with lysostaphin-specific MAb ALS8.1 (18) by an enzyme-linked immunosorbent assay and by immunoblotting. For the enzyme-linked immunosorbent assay, supernatants were mixed with an equal volume of 45 mM sodium carbonate-bicarbonate buffer (pH 9.6) and added to 96-well plates for 2 h at 37°C before blocking with bovine serum albumin (1 mg/ml) and subsequent exposure to ALS8.1. For the immunoblot analysis, samples were electrophoresed, transblotted onto an Immobilon-P polyvinylidene difluoride transfer membrane (Millipore Corp., Bedford, Mass.) as described previously (18), except that the transfer buffer contained only 7.5% methanol, and blocked by exposure to 0.5% Tween 20. The blots were developed in a solution consisting of 20 mg of 3-amino-9-ethyl-carbazide dissolved in 5 ml of dimethyl formamide, 95 ml of 0.05 M sodium acetate (pH 5.0), and 100 μ l of 30% (vol/vol) hydrogen peroxide.

Hybridization with the *end* gene probe. DNA was prepared from staphylococci that had been treated with acetone, lysozyme, and SDS as previously described (22). Hybridization analyses were performed essentially as described by Ausubel et al. (6). DNA was digested with *Eco*RI, transferred to nitrocellulose membranes, and probed with an internal *Ava*II fragment from the lysostaphin gene (*end*) that was gel purified (36) and [α -³²P]dCTP labeled with a random primed DNA labeling kit (U.S. Biochemical Corp., Cleveland, Ohio) according to the manufacturer's instructions. The results were visualized by autoradiography.

RESULTS AND DISCUSSION

Normalized ribotype patterns. Normalized ribotyping provides an approach in which a species can be viewed as an assembly of known pattern types, in contrast to conventional phenotypic typing, in which a species is divided into morphotypes and/or biotypes. When *S. sciuri* strain patterns were assembled, they could be arranged into three subdivisions or blocks on the basis of similarities (Fig. 1). The block of pattern types bounded by dd 4535 and dd 4376 in Fig. 1 represents the patterns of *S. sciuri* subsp. *rodentium*. These patterns are distinguished by a band at about 8.5 kbp. There is also another unique band at about 4.8 kbp that has a high frequency of occurrence. In the two pattern types without this band, there are additional polymorphic fragments which might contain the same hybridizable region. In the block representing the strains of *S. sciuri* subsp. *carnaticus*, the patterns are distinguished by a dark band at 12 kbp, suggesting that there is more than one hybridizable fragment of this size. The lower intensity of this band in pattern dd 0791 could be accounted for by the band at about 6 kbp. The block of patterns at the top of Fig. 1 represents the pattern types of *S. sciuri* subsp. *sciuri*. The patterns of this subspecies could be distinguished either by the high intensity of a band at about 4.2 kbp that had a high frequency of occurrence or by the presence of two separable bands at about

FIG. 1. Normalized ribotypes for *S. sciuri* strains and the nomenclatural type strains of the other species in the *S. sciuri* species group. The patterns delineate three subspecies of *S. sciuri*, with taxonomic resolution extending to the level of type. Patterns dd 4532 to 4731 are *S. sciuri* subsp. *sciuri* patterns. Patterns dd 0791 to 4750 are *S. sciuri* subsp. *caraticus* patterns. Patterns dd 4535 to 4376 are *S. sciuri* subsp. *rodentium* patterns. Pattern dd 0756 is the pattern for the nomenclatural type strain of *S. vitulus*, and pattern dd 4201 is the pattern for the nomenclatural type strain of *S. lentus*.

4.2 and 4.3 to 4.5 kbp. When an array of patterns is accumulated for a species, similarities of pattern types can be recognized and subspecies can be defined. The separation of *S. sciuri* into three subpopulations or subspecies by ribotyping correlated well with the results of other methods described below.

Pattern type dd 4201, representing the nomenclatural type strain of *Staphylococcus lentus*, and pattern type dd 0756, representing the nomenclatural type strain of *Staphylococcus vitulus*, two other species of the *S. sciuri* species group, are visually distinct from the patterns obtained for *S. sciuri*. While all data

TABLE 2. Results of hybridization of staphylococcal DNAs with [methyl-³H]thymidine-labeled DNAs from the *S. sciuri* subspecies

Species or subspecies	Ribotype pattern type	Strain	% Relative binding with labeled DNA from:					
			<i>S. sciuri</i> subsp. <i>sciuri</i> strains			<i>S. sciuri</i> subsp. <i>camaticus</i> strains		<i>S. sciuri</i> subsp. <i>rodentium</i> DD 4761 ^T (70°C)
			DD 4277 ^T		DD 4537 (70°C)	DD 791 ^T (70°C)	DD 6081 (70°C)	
			55°C	70°C				
<i>S. sciuri</i> subsp. <i>sciuri</i>	dd 0868	DD 4277 ^T	100^a	100	80	64	63	58
	dd 0868	DD 4274		92	81	60	59	65
	dd 4281	DD 4281		98	81			
	dd 4289	DD 4289		100	80	60	59	57
	dd 4532	DD 5748	84	88	79	64	61	60
	dd 4292	DD 4537	91	83	100	62	51	63
	dd 4292	DD 5256		80	82	67	61	57
	dd 0823	DD 6059		84	90			
	dd 4731	DD 4731		83	98	66	58	56
dd 6044	DD 6044		85	90	62	65	55	
<i>S. sciuri</i> subsp. <i>camaticus</i>	dd 0791	DD 791 ^T	68	68	66	100	90	63
	dd 0791	DD 5284		67	65	85	81	68
	dd 0791	DD 5254	73	70	61	81	98	65
	dd 4750	DD 6081	72	60	69	89	100	67
	dd 4750	DD 4750		73	64	98	86	61
	dd 4530	DD 4530		72		81		64
	dd 4530	DD 5666		61		99		61
<i>S. sciuri</i> subsp. <i>rodentium</i>	dd 4761	DD 4761 ^T	67	64	68	65	54	100
	dd 4761	DD 5282						80
	dd 4535	DD 4535	74	51	63	65	53	88
	dd 4758	DD 6077	74	49	58	54	62	86
<i>S. vitulus</i>	dd 0756	DD 756 ^T		18	15	14	14	14
<i>S. lentus</i>	dd 4201	DD 4203 ^T		15	13	10	10	14

^a The values in boldface type are intrasubspecies values.

are not shown, sets of fragments that are conserved in different strains of a particular species are not conserved as a set in the strains of any other of the more than 200 species studied to date (11, 12, 23, 59).

DNA-DNA hybridization studies. The DNA relationships among selected *S. sciuri* strains representing the major ribotype pattern types, several minor types, and other species of the *S. sciuri* species group are shown in Table 2. The data show that each proposed subspecies forms a separate, well-defined DNA similarity group at the stringent criterion (70°C). Strains of the same subspecies exhibited high levels of DNA relatedness (mean relative level of binding in reassociation reactions, 87%; range, 79 to 100%), whereas, strains of different subspecies exhibited significantly lower levels of DNA relatedness (mean relative level of binding, 62%; range, 49 to 73%). The levels of DNA relatedness of three representative strains of each subspecies to *S. sciuri* subsp. *sciuri* determined under the optimal conditions (55°C) (mean relative level of binding, 71%; range, 67 to 74%) support the conclusion that these similarity groups should be given subspecies status rather than species status. Nevertheless, the extents of DNA divergence between subspecies appear to be quite large and suggest that the subspecies are approaching species status.

Phenotypic characterization. Cells were gram-positive cocci (diameter, 0.7 to 1.2 µm), that were nonmotile, did not form spores, and were arranged singly, in pairs, and in tetrads. *S. sciuri* is a facultative anaerobe, although growth is much better under aerobic conditions. All strains were positive for catalase, oxidase (as determined by the Microdase disk test), and β-glu-

cosidase activities and esculin hydrolysis and produced acid aerobically from D-glucose, glycerol, β-D-fructose, sucrose, and D-ribose. All strains except strain DD 4537 reduced nitrate to nitrite. All strains were negative for staphylocoagulase, thermonuclease, pyrrolidonyl arylamidase, ornithine decarboxylase, urease, β-glucuronidase, and β-galactosidase activities and arginine utilization and failed to produce acid aerobically from raffinose, xylitol, and melibiose and anaerobically from D-mannitol. All strains except strain DD 5748 failed to produce acid aerobically from N-acetylglucosamine. All strains were borderline negative or weakly positive in the STAPH Trac Voges-Proskauer test for acetoin. However, in the ID32 STAPH Voges-Proskauer test and standard methyl red-Voges Proskauer medium, all strains except DD 4289 were negative; strain DD 4289 was weakly positive. Characteristics that were variable for *S. sciuri* are listed in Table 3. Several of the phenotypic characteristics were expressed differently in the different subspecies and, therefore, had some discriminatory value. For example, a high percentage of the strains of *S. sciuri* subsp. *rodentium* gave moderate to strong positive reactions in the latex agglutination test for clumping factor and protein A, whereas the strains of *S. sciuri* subsp. *sciuri* were negative in this test. All strains of *S. sciuri* subsp. *camaticus* produced acid from D-xylose but failed to produce acid from D-melezitose, and all strains of *S. sciuri* subsp. *sciuri* produced acid from D-cellobiose.

Antibiotic resistance. Breakpoints established for *S. aureus* were used for classifying *S. sciuri* strains as "susceptible", "intermediate resistant", or "resistant" to all of the antibiotics tested except novobiocin. All strains of *S. sciuri* were resistant

TABLE 3. Variable characteristics of *S. sciuri* subspecies

Characteristic	% of strains positive (% of strains weakly positive)		
	<i>S. sciuri</i> subsp. <i>sciuri</i> (n = 26)	<i>S. sciuri</i> subsp. <i>camaticus</i> (n = 14)	<i>S. sciuri</i> subsp. <i>rodentium</i> (n = 18)
P-agar colony diam $\geq$ 7 mm	65	7	33
Pigment (cream-yellow, yellow, or orange-gray)	69	57	44
Anaerobic growth	23 (62)	0 (64)	0 (44)
Hemolysis (bovine blood)	8 (92)	0 (100)	0 (100)
Staph Latex agglutination	0	36	94
Staph-A-Lex agglutination	0	0	89 (6)
Alkaline phosphatase activity	85 (8)	29	67
Staphylolytic activity	88 (12)	71 (14)	77 (11)
Oxacillin resistance ^a	27 (38)	7 (29)	56 (33)
Methicillin resistance ^b	4 (38)	0 (14)	22 (44)
Cefazolin resistance ^c	0	0	0 (28)
Acid produced aerobically from:			
D-Xylose	12	93 (7)	17
D-Melezitose	54 (8)	0	44
D-Cellobiose	88 (12)	29 (29)	56 (11)
D-Sorbitol	77	71 (7)	28 (6)
L-Arabinose	38 (15)	14 (7)	22 (33)
D-Mannose	65 (19)	64 (21)	83 (17)
α -Lactose	15	29	0
D-Fucose	92 (4)	100	67
D-Trehalose	81 (12)	93 (7)	56 (44)
Maltose	15 (35)	50 (50)	17 (72)
D-Mannitol	96 (4)	93	100
Salicin	69 (8)	86	89
α -L-Rhamnose	12	0	6

^a Diameters of zones of inhibition for 1- μ g discs were interpreted as follows: 6 to 10 mm, resistant (positive); 11 to 12 mm, intermediate resistance (weakly positive).

^b Diameters of zones of inhibition for 5- μ g discs were interpreted as follows: 6 to 9 mm, resistant (positive); 10 to 13 mm, intermediate resistance (weakly positive).

^c Diameters of zones of inhibition for 30- μ g discs were interpreted as follows: 6 to 14 mm, resistant (positive); 15 to 17 mm, intermediate resistance (weakly positive).

to novobiocin on the basis of breakpoints established for *Staphylococcus saprophyticus* (29). All strains were also resistant to penicillin G, although there was significant variation (10-U disk inhibition zone diameters, 10 to 28 mm) in the level of resistance. Most strains were susceptible to tetracycline, erythromycin, and clindamycin. Strains DD 5262, DD 4780, DD 4750, and DD 5238 were resistant to tetracycline. Strain DD 791^T (T = type strain) was resistant to erythromycin. Strains DD 4776 and DD 792 were multiply resistant to tetracycline, erythromycin, and clindamycin. All strains were susceptible to vancomycin. Most strains were susceptible to gentamicin and sulfamethoxazole-trimethoprim. Strains DD 4371, DD 6272, DD 6273, DD 6278, DD 6281, and DD 6282 were resistant to gentamicin. Strains DD 792 and DD 6276 were resistant to sulfamethoxazole-trimethoprim. Strains exhibited either intermediate resistance (83% of the strains) or near-intermediate resistance (17% of the strains) to ciprofloxacin, and this property may be related to the intrinsic resistance of the organisms to novobiocin and nalidixic acid, other antibiotics that inhibit DNA gyrase activity.

Many strains of *S. sciuri* were either resistant or showed intermediate resistance to oxacillin and methicillin, and some strains showed intermediate resistance to cefazolin. The frequencies and levels of resistance to these antibiotics were gen-

erally higher in *S. sciuri* subsp. *rodentium* than in the other subspecies (Table 3). The diameters of the halos for oxacillin (1- μ g disc), methicillin (5- μ g disc), cefazolin (30- μ g disc), and cephalothin (30- μ g disc) for the subspecies were as follows: *S. sciuri* subsp. *sciuri*, 12 $\pm$ 4, 14 $\pm$ 3, 26 $\pm$ 3, and 29 $\pm$ 3 mm, respectively; and *S. sciuri* subsp. *camaticus*, 14 $\pm$ 3, 15 $\pm$ 3, 27 $\pm$ 3, and 29 $\pm$ 3 mm, respectively; and *S. sciuri* subsp. *rodentium*, 10 $\pm$ 4, 12 $\pm$ 5, 21 $\pm$ 5, and 24 $\pm$ 4 mm, respectively. For many strains, colonies were observed in the halos produced by the oxacillin and methicillin discs. *S. sciuri* subsp. *rodentium* DD 4535, DD 6272, DD 6273, and DD 6282, which exhibited the highest levels of oxacillin and methicillin resistance, produced growth up to the edges of the discs. In the case of *S. aureus*, the inhibition zones included a wide range of MICs, from MICs characteristic of susceptible and/or borderline resistant strains to MICs of highly oxacillin-resistant strains. The mechanisms of oxacillin (and methicillin) resistance in these strains is not yet clear. Chromosomal DNAs prepared from all 40 *S. sciuri* strains tested (20 *S. sciuri* subsp. *sciuri* strains, 6 *S. sciuri* subsp. *camaticus* strains, and 14 *S. sciuri* subsp. *rodentium* strains) gave positive signals with a *mecA*-specific DNA probe, suggesting that a gene closely related to the *mecA* gene of methicillin-resistant strains of *S. aureus* and certain other staphylococcal species was present (5, 47, 56). The results of dot blot hybridization of 20 selected strains are shown in Fig. 2. The fact that all of the strains of *S. sciuri* tested hybridized with the *mecA* gene probe raises the possibility that *S. sciuri* may be an important reservoir of genetic determinants of methicillin resistance. Several other investigators have observed hybridization of a *mecA*-specific DNA probe with the chromosomal DNAs of a few isolates of *S. sciuri* (39, 48, 54). A preliminary report (13) indicated that a homolog of the regulatory gene *mecI/mecR* was also detected recently in a group of *S. sciuri* subsp. *rodentium* strains taken from the present investigation. One attractive hypothesis to consider is that a homolog of the *mecA* gene is intrinsic to *S. sciuri* and encodes one of the penicillin-binding proteins of this species (25, 42). A study of the genetic interactions of *S. sciuri* with other members of the microbial community would be an

FIG. 2. Dot blot hybridization of *S. sciuri* lysates with the *S. aureus* *mecA* probe. The approximate amounts of DNAs loaded on the membrane are indicated in parentheses below. Samples A1 to A11 were lysates of *S. sciuri* subsp. *sciuri* DD 4281 (32 μ g), DD 4278 (37 μ g), DD 4295 (43 μ g), DD 4292 (39 μ g), DD 4276 (36 μ g), DD 4274 (37 μ g), DD 4285 (41 μ g), DD 4289 (45 μ g), DD 4279 (10 μ g), DD 4277^T (34 μ g), and DD 4537 (39 μ g), respectively; samples B1 to B8 were lysates of *S. sciuri* subsp. *rodentium* DD 6272 (16 μ g), DD 6273 (38 μ g), DD 6278 (37 μ g), DD 6281 (43 μ g), DD 6282 (43 μ g), DD 4535 (39 μ g), DD 4761^T (42 μ g), and DD 5282 (39 μ g), respectively; and sample B9 was a lysate of *S. sciuri* subsp. *camaticus* DD 792 (37 μ g). Reference sample C1 was a lysate of methicillin-resistant *S. aureus* COL (30 μ g); sample C3 was a lysate of methicillin-susceptible *S. aureus* RN 2677 (30 μ g); and sample C5 was a lysate of *S. aureus* N315 (30 μ g).

FIG. 3. Production of extracellular staphylytic enzyme activity by colonies grown on an indicator plate containing heat-killed cells of *S. aureus* ATCC 6538P. After growth for 24 h, the colonies were washed from the plate to allow easier visualization of the zones of bacteriolysis. Spot 1, *S. sciuri* subsp. *sciuri* DD 4277^T; spot 2, *S. sciuri* subsp. *camaticus* DD 5255; spot 3, *S. sciuri* subsp. *sciuri* DD 4520; spot 4, *S. sciuri* subsp. *sciuri* DD 5262; spot 5, *S. sciuri* subsp. *rodentium* DD 6282; spot 6, *S. sciuri* subsp. *sciuri* DD 4747; spot 7, *S. simulans* biovar staphylyticus NRRL B-2628.

important step toward understanding the mechanisms of gene flow from this primitive, commensal species to the more specialized, pathogenic species of medical interest.

Staphylytic enzymes. All strains of *S. sciuri* subsp. *sciuri*, 11 of 14 *S. sciuri* subsp. *camaticus* strains (79%), and 16 of 18 *S. sciuri* subsp. *rodentium* strains (89%) exhibited extracellular staphylytic activity when a lysis plate test was used. The staphylytic activities of several representative strains are shown in Fig. 3. Staphylytic activity was detected in all six *S. lentus* strains and 4 of the 11 *S. vitulus* strains tested, but it was observed infrequently in the other staphylococcal species indigenous to lower mammals. This activity is at least in part due to the production of a staphylytic enzyme that is immunologically related to lysostaphin (Table 4), an endopeptidase that has as its main target the polyglycyl bridge of the cell wall peptidoglycan of staphylococci (55). The staphylytic activity profiles and an immunoblot with lysostaphin-specific MAb of selected strains are shown in Fig. 4. Even though most strains reacted with the lysostaphin-specific MAb, only three reactive strains (DD 4747, DD 5262, and DD 6282) hybridized with the *end* gene probe (Table 4). These results suggest that the staphylytic activity of most strains is due to the presence of an enzyme other than lysostaphin that shares an immunological domain or epitope and is determined by a different gene exhibiting little nucleotide sequence homology with *end*. A survey of the total DNAs of many other staphylococcal species indigenous to lower mammals, including *S. vitulus* and *S. lentus* of the *S. sciuri* species group, *Staphylococcus caseolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus delphini*, *Staphylococcus intermedius*, *Staphylococcus schleiferi*, *Staphylococcus felis*, *Staphylococcus simulans*, *Staphylococcus carnosus*, *Staphylococcus gallinarum*, *Staphylococcus equorum*, *Staphylococcus kloosii*, and *Staphylococcus xylosus*, failed to detect any hybridization with the *end* gene probe. Lysostaphin susceptibility was generally inversely related to staphylytic enzyme production (Table 4).

Cell wall composition. Cell wall peptidoglycan and teichoic acid compositions were determined for several selected strains, including *S. sciuri* subsp. *sciuri* DD 4274, DD 4276, DD 4277^T,

DD 4278, and DD 4285 (determined previously [33]), *S. sciuri* subsp. *sciuri* DD 4520 and DD 4526, *S. sciuri* subsp. *camaticus* DD 791^T, and *S. sciuri* subsp. *rodentium* DD 4761^T. The peptidoglycan type of each of the *S. sciuri* subspecies is L-Lys-L-Ala-Gly₃₋₄. This peptidoglycan type is also found in *S. lentus* and *S. vitulus*. Low levels of L-serine (0.1 to 0.4 mol/mol of glutamic acid) were detected in the peptidoglycans of strains DD 4278, DD 4285, DD 4287, and DD 4761^T. The main cell wall teichoic acid type of many *S. sciuri* strains is poly(glycerolphosphate-*N*-acetylglucosaminylphosphate) (19). However, strain DD 4520 has this type only partially (30 to 40%); the remaining types have not been investigated in detail. Strains DD 791^T and DD 4526 have a polyglycerolphosphate type with *N*-acetylglucosamine substitution. Strain DD 4278 has a teichoic acid containing ribitol instead of glycerol (33); strains SC

TABLE 4. Lysostaphin susceptibility, staphylytic enzyme production, and reactivity with lysostaphin-specific MAb and lysostaphin gene probe for selected strains

Organism	Lysostaphin susceptibility ^a	Staphylytic enzyme production (U/mg [dry cell wt])	Reactivity with MAb ^b	Reactivity with gene probe ^c
<i>S. simulans</i> biovar staphylyticus strains				
Wild type	1.0	2.27	+	+
JN351	26.0	<0.02	—	—
<i>S. aureus</i> FDA 209P	23.4	<0.02	—	—
<i>S. sciuri</i> subsp. <i>sciuri</i> strains				
DD 4277 ^T	10.8	0.23	+	—
DD 4287	6.2	0.36	+	—
DD 4520	19.8	<0.02	—	—
DD 5277	2.6	0.34	+	—
DD 5272	7.2	0.34	+	—
DD 4747	5.6	0.43	+	+
DD 5262	3.4	0.11	+	+
<i>S. sciuri</i> subsp. <i>camaticus</i> strains				
DD 791 ^T	3.4	0.06	—	—
DD 5254	8.8	0.35	+	—
DD 5268	4.4	0.36	+	—
DD 5255	2.0	0.35	+	—
DD 5238	3.2	0.34	+	—
<i>S. sciuri</i> subsp. <i>rodentium</i> strains				
DD 4761 ^T	3.4	0.37	+	—
DD 4762	3.0	0.31	+	—
DD 4776	4.6	0.32	+	—
DD 4778	5.2	0.34	+	—
DD 5283	4.0	0.33	+	—
DD 6077	5.8	0.32	+	—
DD 6272	4.8	0.36	+	—
DD 6273	4.2	0.36	+	—
DD 6276	4.2	0.35	+	—
DD 6278	2.6	0.34	+	—
DD 6282	2.2	0.16	+	+

^a Rate of lysis (change in optical density at 620 nm in 10 min) of heat-treated cells divided by the rate of lysis of heat-treated cells of wild-type *S. simulans* biovar staphylyticus NRRL B-2628 when standardized suspensions were exposed to 1 U of lysostaphin per ml.

^b As determined by an enzyme-linked immunosorbent assay and immunoblotting.

^c As determined by a Southern hybridization analysis.

FIG. 4. Staphylolytic activity profiles (A) and immunoblot results (B) obtained with lysostaphin-specific MAb for concentrated samples from stationary-phase culture supernatant fluids after SDS-polyacrylamide gel electrophoresis. The positions and molecular masses (in kilodaltons) of protein standards are indicated on the right. Lane 1, *S. simulans* biovar staphylolyticus NRRL B-2628; lane 2, *S. sciuri* subsp. *sciuri* DD 4287; lane 3, *S. sciuri* subsp. *caruaticus* DD 5254; lane 4, *S. sciuri* subsp. *rodentium* DD 6077; lane 5, *S. sciuri* subsp. *caruaticus* DD 5268; lane 6, *S. sciuri* subsp. *rodentium* DD 4761^T; lane 7, *S. sciuri* subsp. *rodentium* DD 6282; lane 8, *S. sciuri* subsp. *sciuri* DD 4520.

225 (33) and DD 4761^T have a polyglycerolphosphate type that is substituted with glucose instead of *N*-acetylglucosamine.

DNA base composition. The G+C contents of the DNAs of selected strains, including *S. sciuri* subsp. *sciuri* DD 4274, DD 4276, DD 4277^T, and DD 4278 (33), *S. sciuri* subsp. *caruaticus* DD 791^T, and *S. sciuri* subsp. *rodentium* DD 4761^T were in a narrow range, 35 to 36 mol%.

Description of *Staphylococcus sciuri* subsp. *sciuri* Kloos, Schleifer, and Smith 1976, 23^{AL} emend. Kloos et al. 1996. The description below is based on the characteristics of 35 strains isolated in 1971 through 1975 and reported on previously (33) and an additional 15 strains isolated in 1992 for this study. Of these 50 strains, 26 (11 of the previously described strains and the 15 new strains) were selected to examine additional phenotypic characteristics not reported on previously, including pyrrolidonyl arylamidase, thermonuclease, ornithine decarboxylase, and staphylolytic enzyme activities, clumping factor and protein A (as determined by latex agglutination), esculin hydrolysis, and oxacillin and methicillin resistance. Colonies grow to 7 ± 2 mm in diameter on P agar and to 10 ± 2 mm in diameter on TSA. A more complete description of colony morphology and pigmentation on P agar is given in the original description of the subspecies (33). Glucose fermentation is weak, and the terminal pH is 5.7 to 6.0. Growth in the anaerobic portion of semisolid thioglycolate medium ranges from no detectable growth to moderate growth, depending on the strain. Low levels of L-lactic acid are produced from glucose (33). Menaquinones are unsaturated and are composed mainly of six isoprenoid units (MK-6) (40). Catalase is produced, and this enzyme is immunologically distinct and not closely related to the catalases of other species (51). Growth is good at NaCl concentrations up to 10%. The optimum growth temperatures are 35°C on P agar and 35 to 40°C on TSA. Growth is absent or very poor at 45°C. Strains exhibit moderate to strong caseinolytic and gelatinase activities, but do not exhibit lipolytic and elastase activities (33). Staphylolytic enzyme activity is weak to moderate. Clumping factor and protein A are not produced. All strains produce acid aerobically from D-cellobiose and D-mannitol. Other characteristics of *S. sciuri* subsp. *sciuri* that are uniformly positive or negative for the species are listed above. The variable characteristics for this subspecies are listed in Table 3. The major API STAPH-IDENT profile is 5710 (92%); the major STAPH Trac profile is 6736150 (69%). The major ribotype pattern types are dd 4292 (39%) and dd

0868 (30%). Fifteen different ribotype patterns have been described (Fig. 1). The G+C content of the DNA is 35 to 36 mol%. This subspecies is widely distributed in nature and may have various mammalian and transient bird hosts, although it is seldom found on humans and apes (27, 28, 31–33).

Amended description of type strain ATCC 29062 (Kloos, Schleifer, and Smith 1976). Strain ATCC 29062 (= SC 116 = DD 4277), which was isolated from the skin of an eastern gray squirrel, is the type strain of *S. sciuri* subsp. *sciuri* (33). It has all of the uniform characteristics of the subspecies described previously and above and, in addition, has the properties listed below.

Spherical cells (diameter, 0.8 to 1.2 µm) that occur singly, in pairs, and in tetrads.

Circular P-agar colonies are 9 to 10 mm in diameter, have moderately undulate edges, are raised with slightly elevated centers, are smooth with glistening surfaces, and are translucent to opaque with a yellow-gray pigment. Colonies on TSA are 10 to 11 mm in diameter.

Facultatively anaerobic. Delayed weak growth occurs in the anaerobic portion of thioglycolate medium.

No hemolysis of bovine, sheep, or horse blood occurs.

Moderate alkaline phosphatase and staphylolytic enzyme activities.

Moderate amounts of acid are produced aerobically from D-melezitose, D-cellobiose, L-arabinose, D-fucose, D-trehalose, salicin, D-sorbitol, and D-mannitol. Weak acid is produced aerobically from maltose and D-mannose. No acid is produced from D-xylose, α-lactose, and α-L-rhamnose.

Intermediate resistance to oxacillin.

Type dd 0868 ribotype pattern.

The cell wall peptidoglycan is L-Lys-L-Ala-Gly₄; the teichoic acid is poly(glycerolphosphate-*N*-acetylglucosaminylphosphate).

The G+C content of the DNA is 36.5 mol%.

Description of *Staphylococcus sciuri* subsp. *caruaticus* subsp. nov. *Staphylococcus sciuri* subsp. *caruaticus* (car.na'ti.cus. Med. L. adj. *caruaticus*, pertaining to meat). The description below is based on the characteristics of 14 strains isolated in 1991 and 1992. Colonies grow to 5 ± 2 mm in diameter on P agar and to 9 ± 1 mm in diameter on TSA. Many strains (50% of the strains) produce small (diameter, 3 to 4 mm) colonies on P agar. Growth in the anaerobic portion of semisolid thioglycolate medium ranges from no detectable growth to weak, de-

layed growth. The optimum growth temperatures are 35°C on P agar and 35 to 40°C on TSA. Growth is absent or very poor at 45°C. Culture growth produces hemolysis (greening) of bovine and horse blood. All strains produce acid aerobically from D-xylose, D-fucose, D-trehalose, and maltose. Acid production from maltose is weak to moderate. Acid is not produced from D-melezitose and α -L-rhamnose. Other characteristics of *S. sciuri* subsp. *camaticus* that are uniformly positive or negative for the species are listed above. The variable characteristics for this subspecies are listed in Table 3. The major API STAPH-IDENT profiles are 4710 (50%), 4000 (21%), and 5710 (21%); the major STAPH Trac profiles are 6736550 (36%) and 6336550 (29%). The major ribotype pattern type is dd 0791 (63%). Five different ribotype patterns have been described (Fig. 1). The G+C content of the DNA is 36 mol%. This subspecies is found mainly living on bovine (artiodactyl) hosts and their meat products.

Description of the type strain. The type strain of *S. sciuri* subsp. *camaticus* DD 791 (= ATCC 700058), which was isolated from a sliced veal leg. It has all of the uniform characteristics of the subspecies described above and, in addition, has the properties listed below.

Spherical cells (diameter, 0.7 to 1.2 μ m) occur singly, in pairs, and in tetrads.

Circular P-agar colonies are 5 to 6 mm in diameter, have moderately undulate edges, have raised edges with depressed centers, are smooth with glistening surfaces, are translucent to opaque, and have sectors that are unpigmented to slightly cream tinted. Colonies on TSA are 8 to 9 mm in diameter.

Facultatively anaerobic. Delayed weak growth occurs in the anaerobic portion of thioglycolate medium.

Partial hemolysis (greening) of bovine and horse blood occurs. No hemolysis of sheep blood occurs.

Moderate alkaline phosphatase activity and weak staphylolytic enzyme activity.

Clumping factor and protein A are not detected by latex agglutination.

Moderate amounts of acid are produced aerobically from D-xylose, D-sorbitol, D-mannose, α -lactose, salicin, D-mannitol, D-trehalose, and maltose. No acid is produced from D-cellobiose and L-arabinose.

Resistant to erythromycin.

Type dd 0791 ribotype pattern.

The cell wall peptidoglycan is L-Lys-L-Ala-Gly₃; the teichoic acid is polyglycerolphosphate-N-acetylglucosamine (polyglycerolphosphate substituted by N-acetylglucosamine).

The G+C content of the DNA is 36.3 mol%.

Description of *Staphylococcus sciuri* subsp. *rodentium* subsp. nov. *Staphylococcus sciuri* subsp. *rodentium* (ro.den'ti.um. L. plur. gen. noun *rodentium*, of rodents). The description below is based on the characteristics of 18 strains isolated in 1992. Colonies grow to 6 $\pm$ 2 mm in diameter on P agar and to 10 $\pm$ 1 mm in diameter on TSA. The concentric ridges formed at the edges of colonies are usually higher and wider than the ridges observed in the other subspecies. Growth in the anaerobic portion of semisolid thioglycolate medium ranges from no detectable growth to weak, delayed growth. The optimum growth temperatures are 35°C on P agar and 35 to 40°C on TSA. Growth is absent or very poor at 45°C. Culture growth produces partial hemolysis (greening) of bovine blood. Some strains (39% of the strains) produce greening of horse blood. All strains produce acid aerobically from D-mannose, D-trehalose, and D-mannitol. Acid is not produced from α -lactose. Other characteristics of *S. sciuri* subsp. *rodentium* that are uniformly positive or negative for the species are listed above. The variable characteristics for this subspecies are listed in

TABLE 5. Characteristics useful for differentiating *S. sciuri* subspecies from one another and other members of the *S. sciuri* species group^a

Characteristic	<i>S. sciuri</i> subsp. <i>sciuri</i>	<i>S. sciuri</i> subsp. <i>camaticus</i>	<i>S. sciuri</i> subsp. <i>rodentium</i>	<i>S. vitulus</i>	<i>S. lentus</i>
P-agar colony diam $\geq$ 7 mm	d	—	d	—	—
Staph Latex agglutination	—	d	+	+	d
Staph-A-Lex agglutination	—	—	+, $\pm$	+	—
Alkaline phosphatase activity	+, $\pm$	d	d	—	($\pm$)
Acid produced aerobically from:					
D-Xylose	(d)	+	d	(d)	($\pm$)
D-Melezitose	d	—	d	—	—
D-Cellobiose	+, $\pm$	d	d	(d)	+
Maltose	(d)	+, $\pm$	(d)	—	d
Raffinose	—	—	—	—	+

^a +, 90% or more of the strains are positive; $\pm$, 90% or more of the strains are weakly positive; +, $\pm$, 90% or more of the strains are positive or weakly positive; d, 11 to 89% of the strains are positive; —, 90% or more of the strains are negative. Parentheses indicate that a response is delayed.

Table 3. The two API STAPH-IDENT profiles are 5710 (67%) and 4710 (33%); the major STAPH Trac profile is 6336150 (72%). The major ribotype pattern type is dd 4761 (75%). Six different ribotype patterns have been described (Fig. 1). The G+C content of the DNA is 35 mol%. This subspecies is found mainly living on rodent hosts.

Description of the type strain. The type strain of *S. sciuri* subsp. *rodentium* is DD 4761 (= ATCC 700061), which was isolated from the skin of a Norway rat. It has all of the uniform characteristics of the subspecies described above and, in addition, the properties listed below.

Spherical cells (diameter, 1.0 to 1.2 μ m) occur singly, in pairs, and in tetrads.

Circular P-agar colonies are 6 to 7 mm in diameter, have moderately undulate edges, are raised, are smooth with glistening surfaces, are translucent, and have a light yellow-gray pigment. Colonies on TSA are 9 to 10 mm in diameter.

Facultatively anaerobic. Delayed weak growth occurs in the anaerobic portion of thioglycolate medium.

Partial hemolysis (greening) of bovine blood occurs. No hemolysis of horse and sheep blood occurs.

No alkaline phosphatase activity and moderate staphylolytic enzyme activity.

Positive latex agglutination test for clumping factor and/or protein A.

Moderate amounts of acid are produced aerobically from D-sorbitol, salicin, D-mannitol, D-trehalose, and D-fucose. Very weak acid is produced from D-cellobiose and maltose. No acid is produced from D-mannose, L-arabinose, D-xylose, D-melezitose, α -lactose, and α -L-rhamnose.

Intermediate resistance to oxacillin.

Type dd 4761 ribotype pattern.

The cell wall peptidoglycan is L-Lys-L-Ala-Gly₃-L-Ser_{0.1}; the teichoic acid is polyglycerolphosphate-glucose (polyglycerolphosphate substituted by glucose).

The G+C content of the DNA is 35.2 mol%.

Distinguishing characteristics. The phenotypic characteristics that are useful for distinguishing *S. sciuri* subspecies from each other and closely related species are summarized in Table 5. *S. sciuri* subsp. *sciuri* can be distinguished from the other subspecies on the basis of its ability to produce acid from D-cellobiose, positive alkaline phosphatase activity, and inability to produce either clumping factor or protein A. *S. sciuri*

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