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Identification and Characterization of Clinical Isolates of Members of the *Staphylococcus sciuri* Group

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A total of 28 staphylococcal isolates from human clinical specimens belonging to the *Staphylococcus sciuri* group were identified and characterized. The API Staph and ID32 STAPH correctly identified *S. sciuri* and *S. lentus* but not *S. vitulinus* strains. Identification to the subspecies level was possible only by a PCR-based method.

Members of the *Staphylococcus sciuri* group are widespread in nature, and they can be isolated from a variety of farm animals, pets, and wild animals, as well as from various food products of animal origin (6, 9, 12, 14, 22, 26). This group is made up of *Staphylococcus sciuri* subsp. *carnaticus*, *Staphylococcus sciuri* subsp. *rodentium*, *Staphylococcus sciuri* subsp. *sciuri*, *Staphylococcus lentus*, and *Staphylococcus vitulinus* (12, 26). *Staphylococcus pulvereri* was a member of the *S. sciuri* group until recently, when it was shown that *S. pulvereri* is only a synonym of *S. vitulinus* (originally *S. vitulus*) (15, 23). Although they are principally associated with animals, members of the *S. sciuri* group may colonize humans, and it has been estimated that they may constitute 0.79 to 4.3% of the total number of coagulase-negative staphylococci isolated from clinical samples (8, 20). However, they have been associated with serious infections such as endocarditis (10), peritonitis (25), septic shock (11), urinary tract infection (20), endophthalmitis (1), pelvic inflammatory disease (21), and, most frequently, wound infections (16, 19). The aim of this study was to compare phenotypic (conventional, API Staph, ID32 Staph) and genotypic (PCR) methods for identification of isolates of the *S. sciuri* group.

A total of 28 isolates belonging to the *S. sciuri* group, recovered from 1998 to 2003 from clinical samples at the Institute of Microbiology, School of Medicine, Belgrade, Serbia, and Regional Hospital Příbram, Příbram, Czech Republic, were analyzed (Table 1). Half of them were isolated from urine samples. Some of these strains have been reported previously (18–21) but not investigated for the characteristics presented in this study. All the isolates were previously identified by conventional methods (5, 12, 18, 26) as *S. sciuri* (23 strains), *S. lentus* (3 strains), or *S. vitulinus* (2 strains).

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Staphylocoagulase (free coagulase) activity was determined with rabbit plasma (Torlak, Belgrade, Serbia) by using the tube method (5). Oxidase activity was determined with oxidase diagnostic tablets (Rosco, Taastrup, Denmark). Novobiocin susceptibility was determined on Mueller-Hinton agar (Oxoid Limited, Basingstoke, Hampshire, United Kingdom) with a disk containing 5 μg of novobiocin (Bioanalyse, Ankara, Turkey). Strains were considered to be resistant to novobiocin if the zone of inhibition was ≤16 mm. Commercial identification kits, namely, API Staph and ID32 STAPH (bioMérieux, Marcy-l'Etoile, France), were used according to the manufacturer's instructions. All the strains were coagulase negative and oxidase positive. In addition, the disk diffusion method with the 5-μg novobiocin disk confirmed that all strains were resistant to novobiocin. However, only three *S. sciuri* strains showed resistance to novobiocin by use of the ID32 STAPH kit. The problem with determination of resistance to novobiocin by ID32 STAPH was also noted by Chesneau et al. (2). Moreover, the identification system in the instruction manual (identification table, version 2.0) indicated that only 26% of *S. lentus* isolates and 43% of *S. sciuri* isolates could exhibit resistance to novobiocin by use of the ID32 STAPH kit. Identification of isolates based on the conventional and commercial methods (API Staph and ID32 Staph) agreed in the identification of 26 out of 28 strains. The commercial methods agreed in the identification of all the *S. sciuri* and *S. lentus* strains, although some discrepancies between results obtained by API Staph versus ID32 STAPH were noted (Table 2). However, two isolates identified as *S. vitulinus* by the conventional method were identified as *Staphylococcus capitis* and *S. sciuri* by API Staph and as *S. capitis* by ID32 Staph. This discrepancy could be attributed to the fact that *S. vitulinus* is not included in the database of these tests. Misidentification of the members of the *S. sciuri* group by commercial identification systems has been reported previously (13, 17). Differentiation of *S. sciuri* from *S. vitulinus* is possible on the basis of utilization of mannose, L-arabinose,

TABLE 1. Isolation and molecular identification of 28 clinical isolates of members of the *S. sciuri* group

Strain no.	Yr of isolation	Specimen[a]	Clinical significance[b]	Identification by PCR amplification of the 16S–23S rRNA intergenic spacer region	Band obtained with species-specific primer for *S. sciuri*
2	1998	Tip of CVC	0	*S. sciuri* subsp. *sciuri*	Yes
3	1998	Skin around CVC	0	*S. sciuri* subsp. *sciuri*	Yes
4	1998	Tip of CVC	0	*S. sciuri* subsp. *rodentium*	Yes
6	2001	Vagina	0	*S. sciuri* subsp. *rodentium*	Yes
7	2001	Vagina	0	*S. sciuri* subsp. *rodentium*	Yes
8	2001	Wound	+	*S. sciuri* subsp. *rodentium*	Yes
9	2000	Urine	0	*S. sciuri* subsp. *rodentium*	Yes
10	2000	Urine	0	*S. sciuri* subsp. *sciuri*	Yes
11	2000	Urine	0	*S. sciuri* subsp. *rodentium*	Yes
12	2001	Urine	0	*S. sciuri* subsp. *sciuri*	Yes
13	2000	Tip of CVC	0	*S. sciuri* subsp. *rodentium*	Yes
14	2000	Cavum Douglasi	+	*S. sciuri* subsp. *sciuri*	Yes
15	2001	Urine	0	*S. sciuri* subsp. *rodentium*	Yes
16	2001	Urine	0	*S. sciuri* subsp. *sciuri*	Yes
17	2001	Urine	0	*S. lentus*	Yes
18	2001	Tip of CVC	Probably 0	*S. sciuri* subsp. *rodentium*	Yes
19	2001	Urine	0	*S. sciuri* subsp. *sciuri*	Yes
20	2001	Vagina	0	*S. sciuri* subsp. *rodentium*	Yes
23	2002	Urine	0	*S. sciuri* subsp. *sciuri*	Yes
177	2002	Urine	0	*S. sciuri* subsp. *rodentium*	Yes
179	2002	Wound	+	*S. sciuri* subsp. *rodentium*	Yes
201	2002	Wound	Probably +	*S. sciuri* subsp. *rodentium*	Yes
292	2003	Wound	Probably +	*S. sciuri* subsp. *rodentium*	Yes
293	2001	Urine	0	*S. lentus*	Very weak
294	2001	Urine	0	*S. vitulinus*	Yes
295	2002	Urine	0	*S. vitulinus*	Very weak
296	2002	Urine	+	*S. lentus*	Very weak
297	2003	Cervix	0	*S. sciuri* subsp. *rodentium*	Yes

[a] CVC, central venous catheter.
[b] 0, no clinical significance; +, clinically significant.

TABLE 2. Biochemical and phenotypic characteristics of 28 members of the *S. sciuri* group

Characteristic	No. of strains of the following species[a] testing positive[b] for the indicated characteristic:			
	S. sciuri subsp. *sciuri* (n = 8)	*S. sciuri* subsp. *rodentium* (n = 15)	*S. lentus* (n = 3)	*S. vitulinus* (n = 2)
Tube coagulase	0	0	0	0
Oxidase	8	15	3	2
Novobiocin resistance (5 μg)	8	15	3	2
Urease	0	0	0	0
Arginine dihydrolase	0	0	0	0
Ornithine decarboxylase	0	0	0	0
Esculin	8	15	3	0
D-Glucose	8	15	3	2
D-fructose	8	15	3	2
D-Mannose	8	15	3	0 (2)
D-Maltose	8	15	3	0
D-Lactose	4 (2w)	9, 1w (9, 2w)	3	0
D-Trehalose	8	15	3	0
D-Mannitol	8	15	3	1
D-Raffinose	0	0	3	0
Nitrate reduction	8	15	3	2
Voges-Proskauer	0	0	0	0 (1)
β-Galactosidase	0	0	0	0
Arginine arylamidase	0	0	0	0
Alkaline phosphatase	8	15	1	0
Pyrrolidonyl arylamidase	0	0	0	0
D-Saccharose	8	15	3	2
N-Acetyl glucosamine	5, 3w (6, 2w)	10, 5w (13, 1w)	2, 1w (3)	0
D-Turanose	8	11, 4w	1, 2w	0
L-Arabinose	4	11	2, 1w	0
β-Glucuronidase	5	4	0	0
D-Ribose	8	15	3	0
D-Cellobiose	8	14, 1w	3	0
Xylitol	0	0	0	0
D-Melibiose	0	0	3	0
Xylose	0	1	3	2
α-Methyl-β-glucoside	0	0	1	0

[a] The number of strains identified by PCR as belonging to each species is given after the species name.
[b] By API Staph and ID32 STAPH. Where discrepancies between results by these two tests were noted (D-mannose, D-lactose, Voges-Proskauer, and *N*- acetylglucosamine), results obtained by API Staph are given in parentheses w, weak reaction.

maltose, and 2-naphthyl phosphate (alkaline phosphatase) as substrates: *S. sciuri* utilizes some or all of these while *S. vitulinus* is unable to utilize any of them (5, 12, 26). However, we noted that production of acid from mannose by *S. vitulinus* could vary depending on the identification system (Table 2). Identification of the *S. sciuri* strains to the subspecies level was not possible on the basis of phenotypic characteristics, since *S. sciuri* isolates of different subspecies showed similar biochemical profiles.

Generally, molecular approaches proposed for the identification of staphylococci can be divided into those based on the detection of species-specific sequences and those based on the detection of sequence variations in ubiquitous elements such as rRNA and tRNA operons or chaperonin-encoding genes (3). To the best of our knowledge, species-specific primers have been published only for *S. sciuri* (of all the staphylococci in the *S. sciuri* group) (7). All the strains were tested by PCR using species-specific primers for *S. sciuri* based on previously published primers and methods (7). Bright bands indicating a positive reaction were obtained for all 23 *S. sciuri* strains (Table 1) and agreed with the identification by API Staph and ID32 STAPH. However, the fact that strong or weak hybridization signals were obtained for all *S. lentus* and *S. vitulinus* strains suggests that this set of primers may not be reliable in the identification of *S. sciuri*. PCR amplification of the 16S–23S rRNA intergenic spacer region was performed in accordance with the protocols previously described by Couto et al. (4) and Shittu et al. (16). This PCR method enabled identification of all isolates to the species or subspecies level. Out of the 28 isolates, 8 strains were identified as *S. sciuri* subsp. *sciuri*, 15 were identified as *S. sciuri* subsp. *rodentium*, 3 were identified as *S. lentus*, and 2 were identified as *S. vitulinus* by the 16S–23S rRNA PCR method.

In conclusion, our study showed that the members of the *S. sciuri* group can be identified and differentiated from other staphylococci from human clinical samples on the basis of the oxidase test. Only the recently described *Staphylococcus fleurettii* is also novobiocin resistant, coagulase negative, and oxidase positive as well (24). It should be noted that this bacterium has not been isolated from clinical samples of humans. However, certain problems could arise in the identification of these bacteria to the species level by use of API Staph and ID32 STAPH, since *S. vitulinus* is not included in the database for these tests.

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