

Nasal Carriage of Methicillin-Resistant and Methicillin-Sensitive Strains of *Staphylococcus sciuri* in the Indonesian Population[∇]

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***Staphylococcus sciuri* strains were unexpectedly cultured from healthy persons and patients from Indonesia during a population-based survey on nasal *Staphylococcus aureus* carriage. Fifty-one *S. sciuri* isolates were further characterized. The *S. aureus mecA* gene was detected by PCR in 22 isolates (43.1%), whereas *S. sciuri mecA* was found in 33 isolates (64.7%). The staphylococcal cassette chromosome *mec* (SCC*mec*) regions of *S. aureus mecA*-positive isolates contained elements of classical *S. aureus* SCC*mec* types II and/or III.**

Staphylococcus sciuri is an oxidase-positive, novobiocin-resistant *Staphylococcus* species that is associated mainly with animals (1, 5, 24, 26). Infection and colonization of humans with *S. sciuri* have been described as rare phenomena (2, 3, 9, 11, 28, 29, 32, 33, 36).

The bacterium has recently gained interest after it was discovered that *S. sciuri* strains ubiquitously carry a genetic element (*S. sciuri mecA*) that is closely related to the *mecA* gene found in methicillin-resistant *Staphylococcus aureus* (MRSA) strains (8, 35). This finding led to the proposal that *S. sciuri mecA* might be the evolutionary origin of the *mecA* element carried by MRSA. In *S. sciuri*, however, the *mecA* gene exists as a silent gene of unknown function, since it does not confer resistance to methicillin. Some *S. sciuri* strains also carry a second copy of the gene, identical to *S. aureus mecA*. Only isolates with both *mecA* genes are phenotypically methicillin resistant (9).

During a population-based survey on nasal *S. aureus* carriage among 3,995 individuals on the island of Java, Indonesia, we unexpectedly cultured *S. sciuri* from both healthy persons and patients (17, 18). In this work, we characterized these isolates, with a focus on their susceptibility to methicillin.

The survey was carried out by culturing nasal swabs on phenol red mannitol agar (PHMA; Becton Dickinson, Heidelberg, Germany), on which *S. aureus* produces yellow col-

onies due to its ability to ferment mannitol. Mannitol-fermenting bacteria were identified to the species level with the Slidex Staph Plus agglutination test (SSP) (bioMérieux, Marcy l'Etoile, France) and the Vitek 2 system (bioMérieux). During the first phase of the study, both SSP-negative and -positive isolates were identified to the species level using the Vitek 2 system. Later, this was performed only with SSP-positive isolates. Additional phenotypic tests for the identification of *S. sciuri* isolates included an oxidase test (BBL DrySlide oxidase; Becton Dickinson) and a novobiocin susceptibility test (30). For confirmation purposes, sequence analysis of the 16S rRNA gene was carried out with 13 randomly chosen isolates using primers EUB-L (5'-CTTTACGCCCA[AG]T[AG]A[A T][TCCG-3') and EUB-R (5'-AGAGTTTGATC[AC]TGG [CT]TCAG-3').

Methicillin susceptibility testing was performed by cefoxitin disk diffusion, according to the CLSI criteria (7). Antimicrobial susceptibility of additional antibiotics was determined using the Vitek 2 system (card AST-P549).

Molecular typing of the isolates was performed by pulsed-field gel electrophoresis (PFGE), as described previously for *S. aureus* (16, 27). The presence of the *S. aureus mecA* and *S. sciuri mecA* genes was determined as described previously (9, 22). The presence of areas homologous to regions of the *S. aureus* staphylococcal cassette chromosome *mec* (SCC*mec*) types I to VI was examined in a randomly chosen subset of *S. aureus mecA*-positive and *S. aureus mecA*-negative *S. sciuri* isolates. The *S. aureus mecA*-positive isolates were analyzed using the primer sets for detection of loci A to H, as described by Oliveira and de Lencastre (23), and using PCRs for cassette chromosome recombinase (*ccr*) genes (12–14, 19, 23). The *S.*

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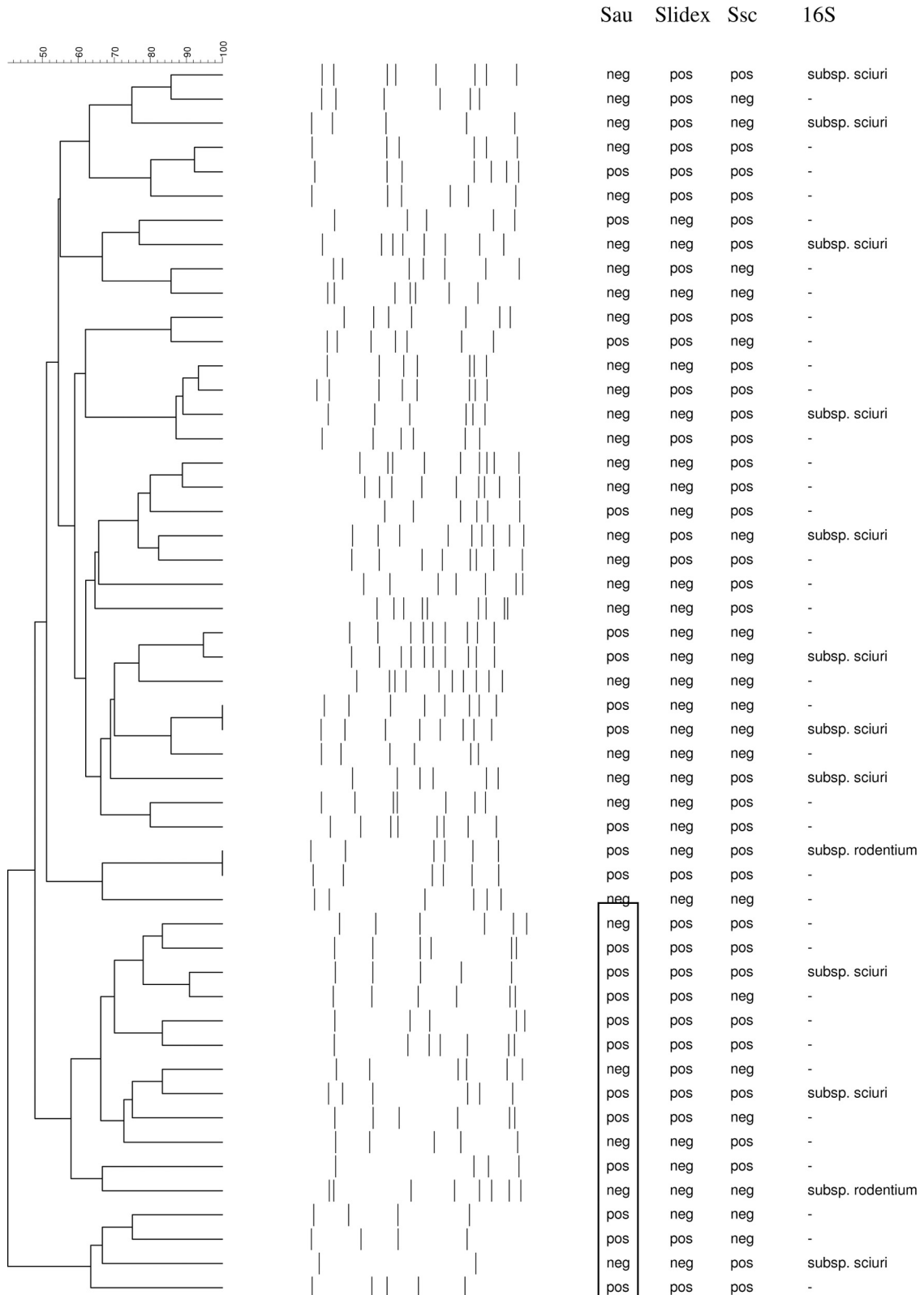


FIG. 1. Dendrogram based on PFGE SmaI restriction pattern analysis of 51 nares-colonizing *S. sciuri* isolates. Similarity analysis was performed with Dice's coefficient, and clustering was done by using the unweighted-pair group method using average linkages (UPGMA) method. The scale at the top shows percentages of similarity. Further information is shown on the right, including the presence (pos) or absence (neg) of the *S. aureus mecA* gene (Sau), positive (pos) or negative (neg) Slidex Staph Plus agglutination test (Slidex) results, presence (pos) or absence (neg) of the *S. sciuri mecA* gene (Ssc), and results of 16S rRNA gene sequencing (16S). The rectangle highlights a clustering of *S. aureus mecA*-positive *S. sciuri* strains.

TABLE 1. Correlation between the presence of the *S. aureus mecA* and *S. sciuri mecA* genes, cefoxitin disk diffusion susceptibility, and antimicrobial susceptibility of 51 isolates of *S. sciuri*

PCR result		No. of isolates with indicated cefoxitin zone diam (mm)		% resistant to ^a :							
<i>S. aureus mecA</i>	<i>S. sciuri mecA</i>	≤24	≥25	ERY	FUS	GEN	NOR	RIF	SXT	TET	VAN
Negative	Negative	0	9 ^b	0	55.6	0	0	0	0	11.1	0
Negative	Positive	0	20	0	60.0	0	0	0	0	5.0	0
Positive	Negative	7	2 ^b	33.3	44.4	55.5	66.7	11.1	22.2	77.8	0
Positive	Positive	12	1	23.1	69.2	38.5	15.4	15.4	7.7	46.2	0
Total		19	32	11.8	58.8	19.6	15.7	5.9	5.9	29.4	0

^a Rates of resistance include resistant as well as intermediate-susceptible isolates, as tested by Vitek 2. Abbreviations: ERY, erythromycin; FUS, fusidic acid; GEN, gentamicin; NOR, norfloxacin; RIF, rifampin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; VAN, vancomycin.

^b These included two *S. sciuri* subsp. *sciuri* isolates.

aureus mecA-negative *S. sciuri* isolates were exclusively subjected to the PCR method of Oliveira and de Lencastre (23).

We found 55 mannitol-fermenting bacteria that were identified as *S. sciuri* bacteria by the Vitek 2 system. Four *S. sciuri* isolates were lost during storage. Thus, 51 isolates were available for further analyses. All 51 isolates were oxidase positive and resistant to novobiocin. Twenty-five strains (49.0%) gave positive SSP agglutination test results. As a check for the phenotypic identification, the 16S rRNA gene from 13 randomly chosen isolates was sequenced, and all appeared specific for *S. sciuri*. Eleven isolates belonged to *S. sciuri* subsp. *sciuri*, and two belonged to *S. sciuri* subsp. *rodentium*. Molecular typing revealed a high degree of genomic diversity among the *S. sciuri* isolates (Fig. 1).

Thus, using PHMA for the detection of mannitol-fermenting bacteria as *S. aureus*, we found a prevalence of nasal carriage of *S. sciuri* in Java, Indonesia, of at least 51/3,995 (1.3%). We did not identify to the species level all SSP-negative staphylococci found in the survey, and the actual prevalence may, thus, be higher. Both *S. aureus* and *S. sciuri* bacteria are mannitol-fermenting bacteria, producing yellow colonies on PHMA. *S. sciuri*, like *S. aureus*, may grow as yellow colonies on blood agar and may give positive SSP and Staphaurex test (Remel) results, which may lead to false species identification (11, 28, 36). The differences between the two species are, among others, novobiocin susceptibility and oxidase and coagulase production. Identification of *S. aureus* by making use of only SSP or the Staphaurex test may result in misclassification of *S. sciuri* as *S. aureus*.

The results of PCR for the *S. aureus* and *S. sciuri mecA* genes, cefoxitin disk diffusion, and antimicrobial susceptibility using Vitek 2 are shown in Table 1. The *S. sciuri mecA* gene was found in 33 isolates (64.7%). Although *S. sciuri* is described to always contain the *S. sciuri mecA* gene, we were unable to demonstrate the presence of the gene in 18 strains (35.3%). False-negative *mecA* PCRs were ruled out by including a 16S rRNA PCR as an internal control. This suggests that a genetic event such as deletion of the gene or mutation or deletion at the primer binding site may have occurred in these isolates. The latter possibility seems more likely, since sequence diversity in the *mecA* homologue of *S. sciuri* has been documented previously (26). On the other hand, the loss of the

putative native *mecA* gene has been reported previously in a strain isolated from a rodent (34). Our findings are supported by the study of Marsou et al., in which they were also unable to detect *S. sciuri mecA* by PCR in 10 out of 30 isolates (20). The *S. aureus mecA* gene was carried by a high percentage of isolates in our collection (43.1%). Similar results were found among isolates obtained from healthy Portuguese carriers (47.8%) and strains obtained from a hospital environment in Serbia (38.1%) (10). Among clinical human isolates and animal isolates, the prevalence was lower, 28.6% and 26.5%, respectively (31). In general, *S. sciuri* strains with both *mecA* genes are oxacillin resistant, and strains without the *S. aureus mecA* gene are oxacillin susceptible (9). This was largely corroborated in the present study. We found three *S. aureus mecA*-positive strains with cefoxitin disk diffusion zones of ≥25 mm. In these strains, the *S. aureus mecA* gene is probably not expressed at significant levels. Such strains have been identified previously (15).

Antimicrobial susceptibility to other antibiotics is shown in Table 1. Overall, the resistance rates of *S. aureus mecA*-positive strains were higher than those of *S. aureus mecA*-negative strains.

Coagulase-negative staphylococci (CoNS) are believed to constitute a reservoir of resistance genes and SCC*mec* elements for *S. aureus*. Therefore, we analyzed the SCC*mec* regions of 18 *S. aureus mecA*-positive *S. sciuri* isolates and 10 *S. aureus mecA*-negative isolates. None of the SCC*mec* regions of the 18 *S. aureus mecA*-positive *S. sciuri* isolates were typeable by the current classification scheme, but most of the strains contained elements of classical SCC*mec* types II (loci C, D, and G) and/or III (*ccr3*) (Table 2). Although we did not search for all elements of SCC*mec* in our isolates, we speculate that SCC*mec* in *S. sciuri* is composed of elements of different classical SCC*mec* types, giving rise to mosaic-like structures. This is in agreement with previous reports and has also been demonstrated for *S. epidermidis* (15, 21). Furthermore, MRSA strains with variable elements in SCC*mec*, possibly originating from CoNS, have been described (25). Loci A to H could not be detected in our *S. aureus mecA*-negative strains, which is in contrast to the report by Juuti et al., in which they describe the presence of loci G and H in 4 out of 7 *S. sciuri* strains (15). When comparing data on SCC*mec* regions of MRSA strains

TABLE 2. SCCmec typing of 18 *S. aureus mecA*-positive *S. sciuri* strains

Profile	No. of isolates	No. of isolates with fox zone diam of ≤ 24 mm ^a	Presence/absence of:													No. of isolates with positive <i>S. sciuri mecA</i> PCR
			Loci described by Oliveira and de Lencastre ^b								<i>ccr</i> loci					
			A	B	C	D	E	F	G	H	<i>ccr1</i>	<i>ccr2</i>	<i>ccr3</i>	<i>ccr4</i>	<i>ccrC</i>	
a	6	3	–	–	+	+	–	–	+	–	–	–	+	–	–	3
b	4	4	–	–	+	–	–	–	–	–	–	–	–	–	–	2
c	3	3	–	–	–	–	–	–	–	–	–	–	–	–	–	1
d	2	2	–	–	+	–	–	–	–	–	–	–	+	–	–	2
e	1	1	–	–	–	+	–	–	–	–	–	–	–	–	–	1
f	1	1	–	–	–	+	–	–	–	–	–	–	+	–	–	0
g	1	1	–	–	–	–	–	–	–	–	–	–	+	–	–	1

^a fox, cefoxitin.

^b Loci described by Oliveira and de Lencastre (31) are as follows: A, downstream of *pls* gene; B, *kdp* operon; C, *mecI* gene; D, *dcs* gene; E, region between pI258 and Tn554; F, region between Tn554 and *orfX*; G, left junction between IS431 and pUB110; and H, left junction between IS431 and pT181.

from Indonesia to those of *S. sciuri* strains, differences and similarities can be noted. Sixty MRSA strains from Indonesia (University of Indonesia, Jakarta, Indonesia) that were analyzed thoroughly by Chongtrakool et al. carried SCCmec type IIIA, including the *ccrC* locus (6). Although parts of the type III cassette were found in our study, *ccrC* was absent in all *S. aureus mecA*-positive strains. The two MRSA strains that were found concurrently in our survey were classified as carrying types III and V by the method of Boye et al. (4). The type III strain was positive for the *ccrC* target, and the type V strain for was positive for both the *ccrC* and *mecA*-IS431 targets. Thus, our data do not support the hypothesis of direct genetic exchange of SCCmec elements between MRSA and *S. sciuri* in this specific setting.

In summary, *S. sciuri* is a colonizer of the nares of people in Indonesia. This bacterial species may be misidentified as *S. aureus*. Further research is needed to investigate the clinical significance of this *Staphylococcus* species in Indonesia. *S. sciuri* may serve as a reservoir of the *S. aureus mecA* gene for *S. aureus*. This potential interaction with *S. aureus* should be investigated as well.

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