

## Identification and Ribotypes of *Staphylococcus caprae* Isolates Isolated as Human Pathogens and from Goat Milk

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**We report five cases of human infection with *Staphylococcus caprae*. Two were community acquired (one case each of endocarditis and urinary tract infection); the other three were acquired in a hospital (two cases of bacteremia associated with intravenous access and one case of urinary tract infection). Analysis of human isolates and goat isolates from eight herds showed that they could be misidentified by some commercial identification systems but were clearly identified as *S. caprae* by ribotyping, according to their species-specific ribotype. Phylogenetic methods applied to the ribotypes did not reveal two distinct lineages corresponding to the goat and human origins of the isolates, although human ribotypes were clearly distinguishable by the presence of a core of four specific bands. The latter observation may reflect some degree of evolutionary change within the species between human and goat isolates.**

The coagulase-negative (CN) species *Staphylococcus caprae* was described in 1983 (6), and *S. caprae* strains have been isolated from goat milk (5, 21) but never from cow or sheep milk (5, 17). Two human clinical isolates of *S. caprae* have been reported: one from the pus of a patient with dermatitis and the other from the urine of a patient hospitalized "with urinary tract infection". There was no convincing evidence that these strains caused true infection (10). *S. caprae* has also been isolated from 6% of 1,500 clinical isolates of CN staphylococci in a Japanese hospital (no further details were given), suggesting that this species can inhabit the human body and is quite widely distributed in hospitals (10). Nevertheless, its actual pathogenic potential for humans has not been clarified. The relationship between human and animal isolates and the existence of cross-contamination between humans and animals has not been established.

We report five cases of human infections with *S. caprae* (one case of infective endocarditis, two cases of intravenous [i.v.] treatment-associated bacteremia, and two cases of urinary tract infection) and compare the bacteriological characteristics and ribotypes of the human isolates with a series of unrelated isolates from goat milk. We showed that human isolates of *S. caprae* were clearly distinguishable by ribotyping of goat isolates, but phylogenetic methods applied to the ribotypes did not produce concordant delineation into two distinct lineages corresponding to the animal and human origins of the isolates.

### CASE REPORTS

**Patient 1.** A 30-year-old woman underwent medullary decompression for a rhabdomyosarcoma in January 1988. The operation was followed by radiotherapy and chemotherapy. In November 1988 the patient was readmitted to the hospital for a relapse of the rhabdomyosarcoma with metastases, and 2 days later she developed a fever (38°C). Her leukocyte (WBC) count was  $3.0 \times 10^9$ /liter. Microscopy of centrifuged urine showed pyuria and cocci. Culture of the urine yielded *S. caprae*

( $10^7$  CFU/ml) in pure growth. The patient was not catheterized. She was given oral cotrimoxazole for 8 days, and although she became afebrile, she died 4 days later from cancer.

**Patient 2.** An 8-year-old previously healthy girl was admitted to a hospital in January 1991 with an abdominal pain and a fever of 40°C that had persisted for 4 days. Her WBC count was  $12 \times 10^9$ /liter with 76% polymorphs, and the erythrocyte sedimentation rate was 109 mm/h. Microscopy of centrifuged urine showed pyuria and cocci, and culture yielded pure growth of *S. caprae* ( $10^7$  CFU/ml). Duplex ureters were found by ultrasonography. The patient was successfully treated for 2 days with i.v. ceftriaxone.

**Patient 3.** A 46-year-old man was admitted to a hospital in August 1992 with a 6- to 8-week history of malaise and night sweats. He had no known cardiac disease and had been deemed fit by a medical examination for diving. On admission, he was febrile (38.2°C), with a hemoglobin level of 12.5 g/liter, WBC count of  $13.3 \times 10^9$ /liter, and erythrocyte sedimentation rate of 80 mm/h. He had no clinical stigmata of endocarditis and was hemodynamically stable. Echocardiography showed a lesion suggestive of an atrial myxoma adjacent to the mitral valve, but since CN staphylococci (later identified as *S. caprae*) were then isolated from 9 of 10 blood culture bottles, the clinicians were persuaded that the "myxoma" was a large vegetation on the mitral valve and decided to excise it. i.v. vancomycin was started then, both as treatment and as prophylaxis for the surgery. The mitral valve was not replaced. The excised vegetation grew *S. caprae*. The patient received a total of 2 weeks of i.v. vancomycin and made an excellent recovery.

**Patient 4.** A 67-year-old woman underwent an ovariectomy for carcinoma in July 1992, and a permanent i.v. device (Portocath) was implanted for chemotherapy. In November, she became febrile (39°C), when chemotherapy was begun. Her WBC count was  $6.7 \times 10^9$ /liter with 69% polymorphs, and the erythrocyte sedimentation rate was 100 mm/h. *S. caprae* was recovered from three blood cultures drawn through the Portocath. The patient was treated with i.v. co-amoxiclav for 2 days followed by oral co-amoxiclav for 8 days. A similar episode occurred in December 1992, and three blood cultures were again positive for *S. caprae*. No blood peripheral cultures were ever taken. The Portocath was removed, and culture of the

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TABLE 1. Origins and characteristics of *S. caprae* strains

Strain	Origin and/or characteristics	Reference and/or source
CCM3573	Reference strain	6
JA21	Clinical, Japan	19, E. Tateda-Suzuki <sup>a</sup>
JA187	Clinical, Japan; <i>mecA</i>	19, E. Tateda-Suzuki
N940142	Goat milk, herd 1	B. Poutrel <sup>a</sup>
N940143	Goat milk, herd 2	B. Poutrel
N940144	Goat milk, herd 3	B. Poutrel
N940145	Goat milk, herd 4	B. Poutrel
N940146	Goat milk, herd 5	B. Poutrel
N940147	Goat milk, herd 6	B. Poutrel
N940148	Goat milk, herd 7	B. Poutrel
N910326	Milk, goat A <sup>b</sup>	Y. Richard <sup>a</sup>
N910327	Milk, goat B <sup>b</sup>	Y. Richard
N920115	Milk, goat C <sup>b</sup>	Y. Richard
N920116	Milk, goat D <sup>b</sup>	Y. Richard
N920117	Milk, goat E <sup>b</sup>	Y. Richard
N920119	Milk, goat F <sup>b</sup>	Y. Richard
N920120	Milk, goat G <sup>b</sup>	Y. Richard
N920121	Milk, goat H <sup>b</sup>	Y. Richard
N880526	Patient 1 (urine)	Hôpital P. Wertheimer <sup>c</sup>
N910072	Patient 2 (urine)	Hôpital E. Herriot <sup>c</sup>
N920271	Patient 3 (cardiac valve)	St. Thomas' Hospital <sup>c</sup>
N930093	Patient 4 (blood culture)	Institut Paoli-Calmettes <sup>d</sup>
N930094	Patient 4 (blood culture)	Institut Paoli-Calmettes
N930095	Patient 4 (indwelling catheter)	Institut Paoli-Calmettes
N930298	Patient 5 (blood culture)	Hôpital L. Pradel <sup>c</sup>
N930299	Patient 5 (blood culture)	Hôpital L. Pradel

<sup>a</sup> See Materials and Methods for location.

<sup>b</sup> Herd 8.

<sup>c</sup> Lyon, France.

<sup>d</sup> Marseille, France.

catheter tip grew *S. caprae*. The patient received a further 10-day course of co-amoxiclav but died 4 months later from cancer.

**Patient 5.** In August 1993, a neonate with aortic coarctation and interventricular communication was transferred from a hospital for ventilation and consideration of surgical treatment. He was treated by dinoprostone to close the aortic coarctation. Four days later, he was extubated, as there was hemodynamic improvement, but within 24 h he became febrile (39°C) and dyspneic with abdominal distension. There were no peripheral pulses, and ventilation was restarted. The WBC count was  $13.5 \times 10^9$ /liter. The umbilical catheter was removed and yielded >100 colonies of *S. caprae* by culture. *S. caprae* was also isolated from two blood cultures. The patient was treated with i.v. vancomycin and amikacin for 8 days followed by oral oxacillin for a further 8 days and made a complete recovery. He later underwent successful surgical treatment of the cardiac abnormalities.

## MATERIALS AND METHODS

**Bacterial isolates.** Four of the above five *S. caprae* infections were reported to the French Centre National de Référence des Staphylocoques in Lyon by clinicians at different hospitals in France between 1988 and 1993. The fifth patient was admitted to St. Thomas' Hospital (London, United Kingdom) with community-acquired endocarditis. The two human clinical isolates of *S. caprae* described by Kanda et al. (10) were kindly provided by E. Tateda-Suzuki and K. Hiramoto (Juntendo University, Tokyo, Japan). Seven *S. caprae* isolates from seven unrelated herds were cultured from the milk of goats with mastitis and were provided by B. Poutrel (Institut National de la Recherche Agronomique, Nouzilly, France). Eight further milk isolates from different goats of the same herd were provided by Y. Richard (Ecole Vétérinaire, Marcy-l'Etoile, France). The patient and animal isolates and the reference strain *S. caprae* CCM3573 (6) were identified and typed in Lyon (Table 1).

**Phenotypic characterization.** Conventional identification of the isolates was performed by using a biochemical gallery (ID32 Staph; bioMérieux, Marcy-

TABLE 2. Results of biochemical tests for patient and goat isolates, strains JA21 and JA187, and the type strain of *S. caprae*, CCM3573

Test <sup>a</sup>	Result for CCM3573	No. positive			
		Patient isolates [n = 8]	Goat isolates [n = 15]	JA21 and JA187	Total [n = 26] (%)
Thermostable DNase <sup>b</sup>	+	8	14	0	23 (88)
Nitrate reduction	+	6	11	2	20 (77)
Urease	-	8	12	2	22 (85)
Arginine dihydrolase	+	8	15	1	25 (96)
Pyrrrolydonyl arylamidase	+	8	12	0	21 (81)
β-Galactosidase	-	0	1	0	1 (4)
Alkaline phosphatase	+	8	15	2	26 (100)
Acetoin production	+	8	15	2	26 (100)
Aerobic acid					
Glucose	+	8	15	2	26 (100)
Fructose	+	8	15	2	26 (100)
Mannose	+	8	14	0	23 (88)
Maltose	-	8	7	2	17 (65)
Lactose	+	4	12	2	19 (73)
Trehalose	+	8	14	2	25 (96)
Mannitol	+	8	10	2	20 (77)

<sup>a</sup> No positive results were obtained for tube coagulase (rabbit plasma), fibrinogen affinity factor (clumping factor), resistance to novobiocin or nitrofurantoin, arginine arylamidase, ornithine decarboxylase, β-glucuronidase, esculin hydrolysis, or aerobic acid from raffinose, sucrose, *N*-acetylglucosamine, turanose, or arabinose.

<sup>b</sup> The positive results for CCM3573, 3 of the 8 patient isolates, and the 14 goat isolates were weak.

l'Etoile, France) (2). The results were interpreted by using both the API database and published references (12). All isolates were tested for coagulase with rabbit plasma (bioMérieux), for fibrinogen affinity factor (clumping factor) by the agglutination procedure (Staphyslide agglutination test; bioMérieux), and for production of heat-stable DNase by a diagnostic kit (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). Hemolytic activity was detected on Trypticase soy agar plates supplemented with 5% defibrinated sheep blood (bioMérieux); test strains were streaked perpendicularly to, but not touching, a streak of a beta-hemolysin-producing strain of *S. aureus* RN4220 (14), and plates were incubated for 24 h at 37°C aerobically. The following characteristics were used to describe the hemolysis observed: alpha-hemolysin produced a wide zone of complete hemolysis with blurred edges, beta-hemolysin gave a wide zone of partial hemolysis which increased after further incubation at 4°C for 4 to 6 h, and delta-hemolysin formed a narrow zone of complete hemolysis and produced a complete clearing within the zone of incomplete hemolysis produced by the beta-hemolysin of *S. aureus* RN4220. The MICs of a range of antistaphylococcal antibiotics were estimated for all strains by an agar dilution technique (16).

**Ribotyping.** Whole-cell DNA was isolated from the staphylococci by standard procedures (18), restricted by *EcoRI* according to the manufacturer's instructions (Boehringer Mannheim, Meylan, France), and separated on a 0.8% agarose gel (Bioprobe, Montreuil-sous-bois, France) in 0.089 M boric acid-0.0002 M EDTA at 2.5 V/cm for 18 h. Subsequently, the DNA was vacuum transferred to nylon membranes (Amersham, Les Ulis, France) and cross-linked with UV light. Hybridization was performed at 60°C with a commercially available 16S-23S rRNA probe labeled with acetylaminofluorene (Eurogentec, Liège, Belgium). Immunoenzymatic detection was carried out according to the manufacturer's instructions (Eurogentec). Raoul I (Appligène, Illkirch, France) was used as the size marker in all blots. Migration distances of the hybridizing bands were measured and were kindly analyzed by P. A. D. Grimont using the Taxolab software (Institut Pasteur, Paris, France). The Dice coefficient (7) was used to compare rRNA gene profiles by scoring positive or negative matches for all bands, and clustering was based on the unweighted pair group arithmetic average algorithm (UPGMA).

## RESULTS

**Microbiological findings.** Strains were identified as *S. caprae* if they were gram-positive, catalase-positive, oxidase-negative cocci, and if the results of the biochemical tests were characteristic of *S. caprae* (Table 2). Twenty-three isolates were identified as *S. caprae* by the API database, one isolate was identified as *S. intermedius* because of the production of β-ga-

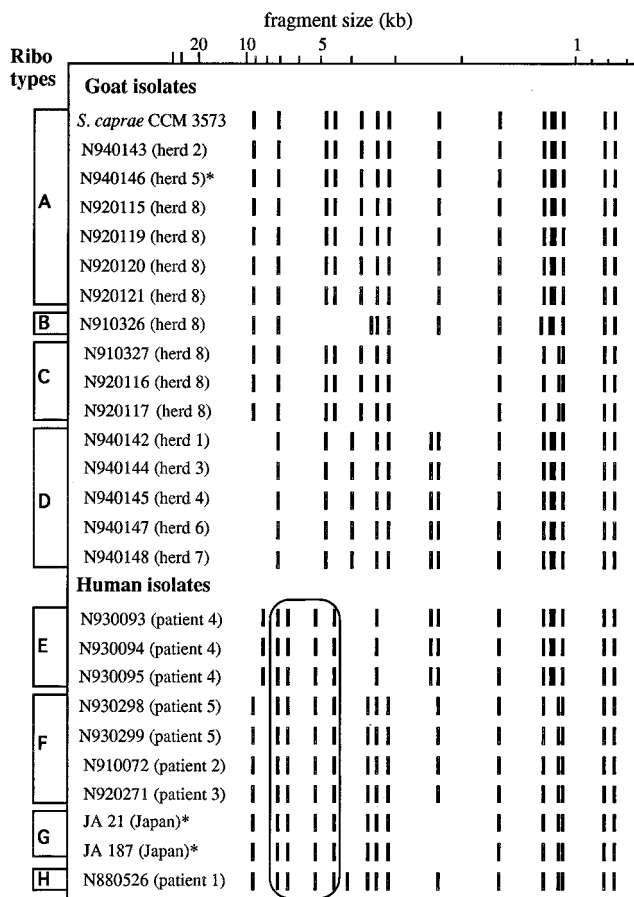


FIG. 1. Schematic representation of the eight *Eco*RI ribotypes (A to H) for the 26 isolates of *S. caprae* studied. \*, species could not be determined with the ID32 Staph gallery. The four bands common to all human isolates are outlined.

lactosidase, and the two isolates from Japan could not be identified. Atypical results for one to three tests (usually mannitol acidification) were indicated by the computer program for most of these isolates. An excellent ( $\geq 99.9\%$ ) or a very good ( $\geq 99\%$ ) identification rate was given by the API system for 13 strains only. Examination of the biochemical results according to Kloos' recommendations (12) and analysis of the ribotypes (see below) definitely assigned all 26 isolates to the group of *S. caprae*. Results of hemolysin assays showed that none of the isolates produced a beta-like hemolysin but 11 isolates (3 of 10 human isolates and 8 of 16 animal isolates) produced an alpha-like hemolysin and 20 isolates (8 of 10 human isolates and 12 of 16 animal isolates) produced a delta-like hemolysin. A delta-like hemolysin was also detected for the reference strain *S. caprae* CCM3573.

The MICs of the antibiotics tested were usually low for human as well as for animal isolates. All isolates were resistant to fosfomycin and susceptible to the other antibiotics, except for the isolates from patients 3 and 5, which were resistant to penicillin by production of  $\beta$ -lactamase.

**Ribotyping.** The 26 isolates analyzed produced eight distinct *Eco*RI ribotypes, designated A to H, consisting of 13 to 16 hybridizing bands, with six being common to the eight patterns (Fig. 1). The ribotype of the reference strain CCM3573 contained all the bands previously observed by de Buyser et al. for the same strain with a 16S rRNA-specific probe (4). Since we

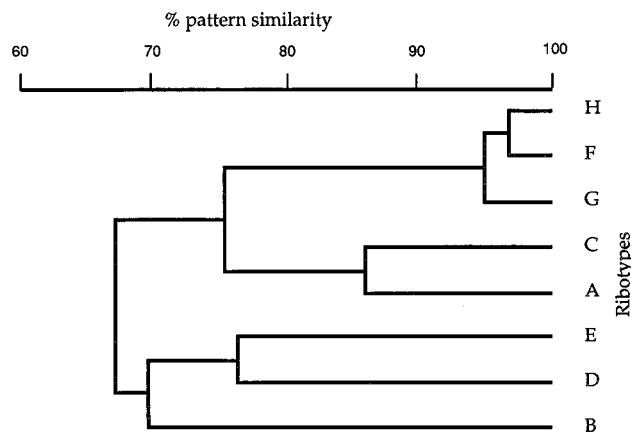


FIG. 2. Dendrogram based upon UPGMA analysis of eight ribotypes of *S. caprae* isolated from goats (A to D) and humans (E to H).

used a 16S-23S rRNA-specific probe, the additional bands present in our case can be attributed to hybridization to 23S rRNA gene fragments. It is interesting that isolate N940146, which could not be identified by conventional methods, showed a ribotype identical to that of the reference strain (ribotype A). Goat isolates produced four distinct ribotypes (A to D) which were all distinct from those observed with the human isolates (E to H). These human ribotypes were easily distinguishable from the goat ribotypes by a core of common bands: two bands of 6.6 and 5.2 kb, strictly specific to the human profiles, and two bands of 7.2 and 4.5 kb also present in some goat ribotypes (Fig. 1). Ribotypes E, G, and H were associated with isolates from patient 4, Japanese patients, and patient 1, respectively. Ribotype F was common to isolates from patients 2, 3, and 5. Among the goat isolates, certain ribotypes (B and C) were observed with isolates from one herd only (herd 8) while other ribotypes (D and A [the ribotype of the reference strain CCM3573]) were scattered among different herds (herds 1, 3, 4, 6, and 7 and herds 2, 5, and 8, respectively).

The UPGMA algorithm was applied on a matrix of Dice's coefficient to estimate the phylogeny of the eight ribotypes. The resulting dendrogram (Fig. 2) did not show a clear-cut distinction between human and goat ribotypes. Ribotype E (patient 4) appeared closer to the goat ribotype D than to the other human ribotypes. Conversely, human ribotypes F, G, and H formed a homogeneous cluster related to ribotypes A and C from goats.

## DISCUSSION

Since the 1960s, certain species of CN staphylococci, especially *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus*, have emerged as pathogens (13). Other, less frequently isolated species have recently been recognized as important pathogens, for instance *S. lugdunensis* in infective endocarditis (22). Although *S. caprae* has been occasionally isolated from human clinical specimens (10), minimal information has been available about these patients and it is not possible to know whether such isolates represent true clinical infection. This study has unequivocally demonstrated that *S. caprae* is a human pathogen capable of causing severe clinical disease both in the community and in the hospital. Our patient 3 had the first reported case of definite infective endocarditis caused by *S. caprae*. The diagnosis was based on persistent positive blood cultures and macroscopic evidence of an intraventricular vegetation at sur-

gery from which *S. caprae* was recovered by culture, thus satisfying the Duke criteria for definite diagnosis of endocarditis (8). Like other species of CN staphylococci, *S. caprae* causes bacteremia associated with i.v. devices and it also appears to be a urinary pathogen capable of causing symptomatic infection in the absence of a urethral catheter.

The discovery of human infections with a species of staphylococcus originally isolated from goat milk raised the question of possible transmission of these bacteria from animals to humans. Although none of our five patients was known to have had contact with goats, our observations do not rule out this hypothesis, as phylogenetic methods applied to the ribotypes did not reveal two distinct lineages corresponding to the goat and human origins of the isolates. On the other hand, this result may illustrate the limits of the UPGMA algorithm in obtaining the correct phylogenetic tree on the basis of restriction fragment data, and this model can be inconsistent if the number of independent data analyzed is small and if there is one branch evolving at a faster rate than the others (3). When our data were analyzed with a different algorithm (Ward's algorithm), a different result, which differentiated the goat ribotypes from the human ribotypes (analysis kindly provided by M. Struelens [data not shown]), was obtained. Visual analysis of the ribotypes (Fig. 1) clearly distinguished the human isolates on the basis of a constant four-band pattern, suggesting a recent common ancestor for these strains. Other authors have found that *S. caprae* human isolates can be distinguished from some goat isolates on the basis of *Sma*I macrorestriction profiles and, to some extent, on the basis of their cellular fatty acids (9, 13). These observations suggest a parallel and independent evolution of the goat isolates and the human isolates. Nothing is known about the normal habitat of human strains of *S. caprae*, but circumstantial evidence from the association of *S. caprae* with nosocomial i.v.-access-associated bacteremias strongly suggests that it must be part of the commensal skin flora. Such information requires accurate identification of CN staphylococci in clinical laboratories. Kawamura et al. (11) reported 80 isolates recognized as *S. caprae* by a DNA-DNA hybridization but initially misidentified as *S. haemolyticus*, *S. warneri*, *S. hominis*, and *S. epidermidis* by conventional methods or commercial kits. In our study only the two isolates from Japan and one goat isolate were not correctly recognized by the API database. The major discrepancy was observed with mannitol acidification, which was positive for 77% of our isolates (Table 2), contrasting with 1% positivity in the API database. When conventional methods for identification were not convincing, the ribotyping was of considerable help since the taxon-specific pattern of *S. caprae* (1, 4) was recognized for these isolates.

When our data are compared with those reported by Kloos (12) and the API database, key characters which help with the identification of *S. caprae* can be defined. *S. caprae* was CN, fibrinogen affinity factor negative, novobiocin susceptible, alkaline phosphatase positive, acetoin positive, and sucrose negative. The thermostable DNase was often positive (88%) but usually weak. All the *S. caprae* strains described by Devriese et al. (6) produced weak hemolysis on sheep blood agar similar to the effect of the *S. aureus* beta-hemolysin. We, however, found that even the hemolysis detected with the reference strain had a synergistic effect with the beta-hemolysin of *S. aureus* RN4220 and was considered to be of a delta type. Most isolates of *S. caprae* produced a delta-like hemolysin which was sometimes associated with an alpha-like hemolysin.

*S. caprae* isolates were usually susceptible to most anti-staphylococcal antibiotics including penicillin for three of the five patients. All 26 strains were uniformly resistant to fosfo-

mycin, a characteristic not reported previously for *S. caprae*. Resistance to methicillin (shown by the presence of the *mecA* gene and the *mec* regulator genes) has been detected only in Japanese human *S. caprae* strains (15, 19, 20). The 10 isolates from goats studied by Devriese et al. (6) were also largely susceptible to antibiotics, but one was resistant to penicillin, whereas all our goat isolates were penicillin sensitive.

The recognition of *S. caprae* as a human pathogen has probably been thwarted by difficulties in its identification. Even with the current availability of commercial kits, not all clinical laboratories identify CN staphylococci, even strains isolated from severe infections, and "*S. epidermidis*" is the term still widely used to refer to such organisms. A larger collection of human isolates of *S. caprae* should allow a modification of the identification scheme defined by the conventional method or commercial kits. This should lead to a greater understanding of the ecology of *S. caprae* and its clinical significance in human and veterinary medicine.

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