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Human Isolates of *Staphylococcus caprae*: Association with Bone and Joint Infections

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***Staphylococcus caprae* is a coagulase-negative, DNase-positive member of the genus *Staphylococcus* usually associated with goats, but since 1991 a few laboratories have reported isolating the organism from human clinical specimens. We report on the isolation of 14 strains from human specimens and note that 10 strains were obtained from patients with bone and joint infections. Nine of the 10 infections started with traumatic fractures, and the other was a case of mastoiditis. Seven of these 10 infections were in patients with orthopedic prostheses, which appears to be a risk factor. Three of the 14 strains were from transplant patients. For three of the patients, *S. caprae* was the only organism isolated. *S. caprae* may be misidentified because it is not represented in the current MicroScan or Vitek identification systems which are in use in many laboratories, but the organism can be differentiated by a few biochemical tests. *S. caprae* produces positive results for DNase, pyrrolidonyl aminopeptidase, and acid production from mannitol and maltose; it produces negative results for ornithine decarboxylase and tube coagulase.**

Coagulase-negative staphylococci are among the most common bacteria isolated in clinical laboratories. Once regarded as harmless commensal organisms, some are now recognized as pathogens capable of causing both nosocomial and community-acquired infections, particularly in immunosuppressed patients or in individuals who use prosthetic devices (9). Infection with *Staphylococcus epidermidis* in such patients is well documented, and there are reports implicating other coagulase-negative species in serious infections. In the last two decades a number of new species of staphylococci have been described. We report here on the isolation from human clinical samples of 14 strains of *S. caprae*, a species usually associated with goats. A large proportion of the strains were isolated from patients with bone and joint infections associated with orthopedic prostheses. We also present relevant laboratory data which may be useful for the recognition of strains of this species in human material.

MATERIALS AND METHODS

Bacterial strains. The sources of the 14 strains of *S. caprae*, together with relevant clinical information, are presented in Table 1. The strains were isolated during the period from November 1990 to April 1996. All strains were catalase-positive, gram-positive cocci appearing in clusters, and all grew well on P agar containing 6.5% (wt/vol) sodium chloride. They were identified as *S. caprae* by means of a self-educating system which has been described in detail elsewhere (1). Briefly, isolates were subjected to cellular fatty acid analysis with the Microbial Identification System (Microbial ID, Inc., Newark, Del.), as well as 35 biochemical tests. They were assigned to the species only if both identification methods gave the same result.

Cultures of staphylococci were suspended in glycerol citrate solution at −70°C for long-term storage and were routinely subcultured to 5% horse blood agar and incubated in air at 35°C.

The type strain of *S. caprae*, ATCC 35538, was obtained from the American Type Culture Collection, Rockville, Md.

Colonial morphology. All the strains were grown on P-agar plates (11) incubated at 35°C for 72 h, followed by further incubation for 48 h at room temperature. Cultures on brain heart Infusion agar (4311065; BBL), Columbia agar (CM 331; Oxoid, Basingstoke, United Kingdom) with 5% horse blood, and Columbia agar with 5% sheep blood were incubated at 35°C for 48 h.

Biochemical tests. Sensitivity to desferrioxamine (13) was tested on P-agar plates with disks impregnated with 1 mg of Desferal (Ciba-Geigy, Dorval, Quebec, Canada). The disk was placed on the second quadrant of a streaked P-agar plate, and the plate was incubated overnight at 35°C in air.

Tests for bound coagulase and protein A were performed with the Slidex Staph Plus kit (bioMerieux, Marcy l'Etoile, France), according to the manufacturer's instructions. Tests for heat-stable nuclease were performed by the microslide method of Lachica et al. (12).

The strains were also examined by using MicroScan Pos Combo 6 panels (Dade International, West Sacramento, Calif.) and Vitek GPI cards (bioMerieux-Vitek, Hazelwood, Mo.), according to the manufacturers' instructions.

Cellular fatty acid analysis. Organisms taken from storage were subcultured at least twice on blood agar before analysis. For fatty acid analysis, organisms were grown on Trypticase soy agar (11043; BBL) containing 5% sheep blood, and the plates were incubated in air at 35°C for 24 ± 1 h. Analysis of cellular fatty acids was performed as described previously (1) by using the Microbial Identification System. Fatty acid profiles were compared with those in a profile library compiled in the Division of Microbiology laboratory and containing entries for all the recognized aerobic species and subspecies of staphylococci with the exception of the recently described species *S. saprophyticus* subsp. *bovis* (6).

Antimicrobial susceptibility testing. Disk diffusion tests were performed by the standard method of the National Committee for Clinical Laboratory Standards (17). MICs were determined with MicroScan Pos Combo 6 panels and Vitek GPS SB cards (bioMerieux-Vitek) according to the manufacturers' recommendations.

The type strain of *S. caprae*, ATCC 35538, was tested in parallel with all the clinical isolates.

RESULTS

Colonial morphology. Colonies on horse and sheep blood agars at 35°C were white, circular, entire, raised, 1 to 2 mm in diameter after 24 h and approximately 4 mm in diameter after 48 h. Some strains showed a narrow zone of beta-hemolysis extending less than 1 mm from the edge of the colony after 48 h of incubation. On brain heart infusion agar after 48 h colonies were 4 to 5 mm in diameter, circular, raised and often with a flat top, and shiny but often with a matte center. On P agar after 5 days colony diameters were 6 to 10 mm, and the colonies were usually white, circular, entire, opaque, and raised. Colonies of most strains had a flat, shiny top with a broad ring of radial striations leading to a transparent periphery. In some cases tiny microcolonies were visible at the pe-

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TABLE 1. Source of strains and relevant clinical information[a]

Isolate no.	Patient age (yr)	Diagnosis	Orthopedic prosthesis	Immuno-compromised	Site of *S. caprae* isolation	No. of specimens yielding *S. caprae*/ no. examined	Microscopic findings (PC/GPC)	Other isolates (no. of specimens)	Outcome
UHL 2455	36	Fracture; tibial osteomyelitis, septic arthritis	Y	N	Ankle synovium	1/6	Few/−	*S. aureus* (5)	Degenerative arthritis
UHL 2457	54	Fracture; knee sepsis, olecranon swelling	Y	N	Bursa fluid from elbow	1/1	Few/−	None	Cured
UHL 2589	52	Bilateral otitis externa	N	N	Left and right ear drainage	2/2	−/++	*Pseudomonas aeruginosa* (2), coliforms (1)	Cured
UHL 3288	44	Fracture; osteomyelitis of calcaneus	N	N	Calcaneus	8/18	+/Few	Anaerobic GPC (11), coliforms (6), *S. aureus* (5)	Unknown
UHL 3741	71	Infected toenail	N	N	Great toe nail	1/1	++/++	None	Unknown
UHL 3745	60	Renal transplant, cytomegalovirus sepsis	N	Y	Nephrectomy site	3/3	+++/Few	*Klebsiella pneumoniae* (1), enterococci (2)	Died
UHL 5214	69	Fracture of malleolus, wound breakdown	Y	N	Ankle	7/11	+/Few	Coliforms (11), *Stenotrophomonas maltophilia* (4)	Cured
UHL 5368	44	Renal transplant	N	Y	Nose	1/1	−/−	*S. aureus* (1)	Died
UHL 5464	33	Fracture of distal tibia-fibula	Y	N	Ankle	5/9	Few/−	*Staphylococcus epidermidis* (2)	Unknown
UHL 5825	69	Fracture; osteomyelitis of ankle	Y	N	Ankle tissue	1/11	Few/−	*Streptococcus anginosus* group (3), anaerobic GPC (4)	Cured
UHL 6259	60	Liver transplant, mastoiditis	N	Y	Left and right ear drainage	2/2	++/+++	None	Died
UHL 6451	68	Medial arthrosis, meniscectomy	N	N	Knee	5/6	++/Few	*S. lugdunensis* (1)	Chronic
UHL 6953	18	Fracture of patella, necrosis	Y	N	Synovial fluid	5/16	+++/−	*S. epidermidis* (5), *Streptococcus mitis* (1)	Unknown
UHL 8603	25	Fracture, tibial osteomyelitis	Y	N	Tibia	5/5	Few/+	Group G streptococci (5), *P. aeruginosa* (5), anaerobic GPC (3)	Cured

[a] All patients were males. Abbreviations: N, no; Y, yes; PC, pus cells; GPC, gram-positive cocci.

riphery. One strain showed pale yellow pigmentation on P agar.

Biochemical tests. All strains gave negative results in tests for oxidase, free coagulase, ornithine decarboxylase, esculin hydrolysis, and acid production from salicin, sorbitol, arabinose, raffinose, cellobiose, xylitol, xylose, and melezitose. None of the strains grew on plates containing novobiocin (1.6 mg/liter) or furazolidone (0.02%), and none was sensitive to desferrioxamine. All strains gave negative reactions when tested for bound coagulase or protein A in the Slidex Staph Plus kit. All strains fermented glucose within 24 h and gave positive reactions in the following tests: arginine hydrolysis, production of DNase (which was not heat stable) and pyrrolidonyl aminopeptidase, and acid production from glucose, trehalose, and fructose. Other tests gave variable results, which are presented in Table 2.

Positive reactions in the gelatinase, Tween 80 lipase, and Voges-Proskauer tests were always weak, whereas DNase tests always gave reactions which were at least as strong as those obtained with an *S. aureus* control strain. Tests for heat-stable nuclease were negative. This is in contrast to results published previously (23), which stated that the DNase was heat stable but usually gave weak reactions. The type strain, ATCC 35538 (a goat strain), was dissimilar to the clinical isolates, giving negative results in the urea, maltose, and Voges-Proskauer tests. Strain UHL 3745 was also unusual in that it was mannose and phosphatase negative. The test for acid production from sucrose was positive for all strains (including the type strain) except UHL 5464. A previous report (4) stated that *S. caprae* strains are negative in this test; this discrepancy is probably due to our use of a more sensitive test.

Cellular fatty acid analysis. All the strains were identified as *S. caprae* by analysis of cellular fatty acids and gave similarity indices of >0.6 against library entries developed in the Division of Microbiology laboratory (1). A similarity index of 0.6 or more represents a good level of confidence in the identification (14). However, the fatty acid profiles were very similar to those for *S. capitis* and *S. haemolyticus*. This means that fatty acid analysis is unable to distinguish reliably between these groups and must be supported by other methods.

Commercially available identification methods. *S. caprae* is not represented in the MicroScan or Vitek databases. The results obtained with both systems are given in Table 3.

Antimicrobial susceptibility tests. The results obtained by the three methods of antimicrobial susceptibility testing were almost completely consistent. However, UHL 3745 fell in the intermediate category when tested against gentamicin by the disk diffusion method but was reported to be sensitive by the commercially available systems. Three strains (strains UHL 3741 and UHL 5214 and the type strain) were sensitive to penicillin G and did not produce beta-lactamase. The other 12 strains were beta-lactamase positive. UHL 3745 was found to be resistant to several of the antimicrobial agents tested, including oxacillin (Table 4). Except for the penicillin resistance noted above, all the other strains were sensitive to all the agents tested. Two penicillin-resistant strains (strains UHL 6953 and UHL 8603) showed small zones of inhibition around the penicillin disk, with small colonies within the zone.

DISCUSSION

S. caprae was first described in 1983 in milk samples taken from healthy goats in France (4). It has since been reported as a commensal organism of goats on several occasions (3, 18, 21). Some strains produce toxic shock syndrome toxin type 1 (22),

TABLE 2. Biochemical tests in which results for 15 strains of *S. caprae* were variable

Test	No. positive/total no. (%)	Strains giving unusual result	% Positive (n = 38)[a]	Comments
Urea hydrolysis	14/15 (93)	ATCC 35538	92	
Lactose acid	4/15 (27)	UHL 2455, UHL 5464, UHL 5214, ATCC 35538	50	
Sucrose acid	14/15 (93)	UHL 5464	95	
Maltose acid	14/15 (93)	ATCC 35538	89	
Mannitol acid	13/15 (87)	UHL 5368, UHL 5825	95	Some weak
Mannose acid	13/15 (87)	UHL 3745, UHL 8603	95	
Galactose acid	8/15 (53)		71	
Nitrate reduction	11/15 (73)	UHL 2589, UHL 3745, UHL 5368, UHL 5825	79	
Phosphatase	14/15 (93)	UHL 3745	92	
Gelatinase	12/15 (80)	UHL 2457, UHL 3745, ATCC 35538	74	Many weak
Tween 80 lipase	5/15 (33)		37	
Acetoin	14/15 (93)	ATCC 35538	87	All weak

[a] Data are from reference 1.

the toxin associated with menstruation-related cases of toxic shock syndrome caused by *S. aureus* (2).

The first report of isolation of *S. caprae* from human sources involved two patients, both of whom had received antibiotics for at least 3 weeks (7). One of the strains was resistant to methicillin (MIC, >100 μg/ml). The investigators stated that in 1990 they isolated 13 additional strains from patients in the same hospital and that this species accounted for 6% of their clinical coagulase-negative staphylococcal isolates. It is now known that some strains possess the *mecA* gene (7, 16, 19, 20), which also determines heterotypic methicillin resistance in *S. aureus.*

Vandenesch et al. (23) reported five cases of human infection with *S. caprae*, including two cases of urinary tract infection, one case of mitral valve endocarditis, and two cases of bacteremia associated with vascular catheters. A 1996 abstract by Morvan and El Solh (15) describes four cases of *S. caprae* infection. Three of these strains were from synovial fluid or tissue from patients with orthopedic prostheses, and the remaining one was from a patient with endocarditis following the implantation of a prosthetic valve.

We report here on the isolation of *S. caprae* from 14 patients, 7 of whom had orthopedic prostheses and one of whom had mastoiditis (Table 1). The 14 strains were isolated during the period from November 1990 to April 1996.

Our first strain of *S. caprae* (strain UHL 2455) was at first not identified because it produced acid from sucrose, mannitol, and fructose, contrary to the original description of the species (4). Subsequent examination of the type strain, strain ATCC 35538, showed that it too gave positive results in these tests by the methods that we used. Valle et al. (21) reported that 16 of 18 strains isolated from goats were fructose positive and that 7 of 18 were mannitol positive. All their strains failed to produce acid from sucrose. Strain UHL 2455 was later included in the study by George and Kloos (5), in which seven strains of *S. caprae*, including UHL 2455, were subjected to DNA-DNA hybridization and their identities were confirmed.

There are no colonial or morphologic features which allow for a rapid distinction between *S. caprae* and many other species of coagulase-negative staphylococci. However, it may be recognized by a combination of biochemical tests, including its strongly positive DNase and pyrrolidonyl aminopeptidase reactions and acid production from maltose. Relatively few human staphylococci give strong reactions in the DNase test, and data for differentiation among them are given in Table 5. Further information may be found in our previous publication (1). Tests for free and bound coagulase and for protein A allow for the easy distinction between *S. caprae* and most strains of *S. aureus.*

The low incidence of *S. caprae* isolation which is suggested by the literature is not due to difficulty in its biochemical identification. It is more likely due to low awareness in clinical laboratories and to its absence from the databases of several of the more commonly used commercially available systems. Lab-

TABLE 3. Results for 15 strains of *S. caprae* obtained with two commercially available identification systems[a]

Strain	MicroScan Pos Combo 6 Panels	Vitek GPI
UHL 2455	303360 *S. carnosus* LS 69.87%	77567020010 *S. simulans* 87% GCMS
UHL 2457	307360 *S. carnosus* LS 72.37%	73565020010 *S. warneri* 87% GCMS
UHL 2589	203320 Rare biotype	73565020010 *S. warneri* 87% GCMS
UHL 3288	307362 *S. haemolyticus* LS 38.72%	73525020010 *S. haemolyticus* 69% GCMS
UHL 3741	307323 *S. aureus* LS 78.47%	73565060010 *S. warneri* 92%
UHL 3745	222302 *S. haemolyticus* LS 80.55%	73565060050 *S. warneri* 96%
UHL 5214	303320 *S. haemolyticus* LS 58.85%	77567020010 *S. simulans* 87% GCMS
UHL 5368	207302 *S. haemolyticus* Good ID 92.50%	77567020010 *S. simulans* 87% GCMS
UHL 5464	307364 *S. epidermidis* VGI 97.35%	77567000010 *S. simulans* 90%
UHL 5825	207362 *S. warneri* LS 52.03%	77567020010 *S. simulans* 87% GCMS
UHL 6259	303362 *S. haemolyticus* LS 71.59%	73565020010 *S. warneri* 87% GCMS
UHL 6451	307320 *S. epidermidis* LS 60.84%	73565020010 *S. warneri* 87% GCMS
UHL 6953	307363 *S. aureus* Good ID 94.45%	73565020010 *S. warneri* 87% GCMS
UHL 8603	313361 *S. aureus* VGI 99.9%	76515020040 *S. warneri* 65% GCMS
ATCC 35538	307300 *S. carnosus* VGI 87.72%	77527020010 *S. haemolyticus* 56% GCMS

[a] LS, low selectivity; VGI, very good identification; GCMS, good confidence, marginal separation.

TABLE 4. MICs of various antimicrobial agents for strain UHL 3745 obtained with MicroScan Pos Combo 6 panels and Vitek GPS SB cards

Antimicrobial agent	MIC (mg/liter)	
	MicroScan Pos Combo 6 panel	Vitek GPS SB card
Amoxicillin-clavulanic acid	8/4	
Ampicillin	>8	
Cefazolin	>16	
Ceftazidime	32	
Cephalothin	16	≥32
Ciprofloxacin	>2	≥4
Clindamycin	>2	
Erythromycin	>4	≥8
Gentamicin	4	≥8
Imipenem	>8	≥16
Nitrofurantoin	≤32	
Norfloxacin	>8	
Oxacillin	>4	≥8
Penicillin G	>8	≥16
Rifampin	≤1	
Tetracycline	≤2	
Trimethoprim-sulfamethoxazole	≤2/38	≤10
Vancomycin	≤2	1

oratories which use such systems may find the profile numbers in Table 3 useful and may confirm an identification of *S. caprae* with a few extra tests.

MicroScan Pos Combo 6 panels often gave low selectivity results (Table 3), but 5 of 14 strains were wrongly assigned to other species. The Vitek system usually gave a "good confidence-low selectivity" comment, but it identified three strains as *S. simulans* or *S. warneri*. Kloos and George (10) included 11 strains (presumably goat strains) in an evaluation of MicroScan Pos ID and Rapid Pos ID panels. They reported correct identifications for 10 strains with the Rapid Pos ID panels but stated that the databases had been modified by the addition of extra data, including data obtained in the study. Obviously, these were not the standard, generally available databases.

Apart from the 14 patients described in Table 1, during the period covered by this study we recognized only one further strain of *S. caprae*, from an external environmental sample. Other isolates of *S. caprae* may not have been identified to the species level because they were not considered significant. In any case, our isolation rate seems lower than that of Kanda et al. (7), who found that *S. caprae* accounted for about 6% of their isolates of coagulase-negative staphylococci.

TABLE 5. Differentiation between *S. caprae* and other DNase-positive staphylococci found in human samples[a]

Staphylococcal species	% Positive				
	Tube coagulase	Ornithine decarboxylase	Mannitol	Maltose	PYR[b]
S. caprae (n = 38)	0	0	95	89	100
S. aureus subsp. *aureus* (n = 133)	99	0	94	100	0
S. capitis subsp. *capitis* (n = 39)	0	0	97	3	0
S. capitis subsp. *ureolyticus* (n = 36)	0	0	97	100	0
S. intermedius (n = 86)[c]	94	0	13	100	99
S. lugdunensis (n = 109)	0	100	1	100	99
S. schleiferi subsp. *schleiferi* (n = 31)	6 (weak)	0	0	0	100

[a] Data are from reference 1.
[b] PYR, pyrrolidonyl aminopeptidase.
[c] Mainly canine strains; data for strains from other animal hosts may differ.

The most striking feature of our series of strains is that 10 of 14 (71%) were associated with bone and joint infections (Table 1). Seven of nine fracture patients were initially treated by open reduction and internal fixation, and fixation hardware appears to be a significant risk factor. One patient had bilateral mastoiditis, with positive radiological findings; the culture yielded only *S. caprae*. All 14 patients in our series were male. This possibly reflects lifestyle risk factors for bone and joint injury. Three patients (21%) were transplant patients. The mortality rate for the immunocompromised patients was 100%, but it is unlikely, on the basis of chart review, that these deaths were directly attributable to *S. caprae* infection.

Table 1 indicates the number of specimens examined from each patient and the number of samples that yielded *S. caprae*. Of 11 patients with multiple specimens, for 9 patients *S. caprae* was isolated from more than one specimen, indicating that the organism was present in the lesion over a period of time. Of eight patients whose direct smears showed gram-positive cocci, four yielded *S. caprae* as the only gram-positive coccus isolated. *S. caprae* was isolated along with well-established pathogens from some patients, and it is impossible to determine its significance in these patients. However, *S. caprae* was the only organism isolated from three patients. Its presence along with numerous pus cells indicates that it is capable of causing infection. For one patient (infected with strain UHL 6451), while *S. lugdunensis* was isolated from one sample, five samples yielded *S. caprae.*

The variety of biochemical and antimicrobial susceptibility patterns among our strains rules out a point source or cross-infection problem within the hospital. While we have no information regarding contact of any of these patients with goats, our strains are biochemically similar to human isolates reported in the literature. Further studies are required to establish the prevalence and distribution of this species in the human body, but it seems likely that the organisms were part of the skin flora of the patients.

The high proportion of patients with orthopedic prostheses strongly suggests an association of this species with bone and joint infections, either as a sole pathogen or in company with other species. It is not known why *S. caprae* should have such a predilection for these sites, and possible virulence factors should be the subject of future studies.

For the reasons stated above, it is quite likely that strains of *S. caprae* are overlooked or misidentified in the clinical laboratory. This possibility was pointed out by Kawamura et al. (8), who identified by DNA-DNA hybridization 80 strains of *S. caprae* which had been earlier misidentified as *S. haemolyticus*, *S. warneri*, *S. hominis*, or *S. epidermidis*. With greater awareness, more case reports of human infection with *S. caprae* will likely arise, and this will permit a better analysis of risk factors. The recognition and identification of *S. caprae* are not difficult, and only by general recognition in routine laboratories will its true pathogenic potential be understood.

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