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Staphylococcus intermedius in Canine Gingiva and Canine-Inflicted Human Wound Infections: Laboratory Characterization of a Newly Recognized Zoonotic Pathogen

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Staphylococcal gingival flora was characterized in cultures from 135 dogs. *Staphylococcus intermedius* was isolated in 39% of the cultures, *S. aureus* was isolated in 10%, and both were isolated in 2.0%. *S. aureus* was isolated more often from dogs of working breeds with weights of >40 lb (ca. 18 kg) and with outdoor habitats than was *S. intermedius*, which was associated with dogs of nonworking breeds with weights of <40 lb and indoor habitats. *S. intermedius* was distinguished from *S. aureus* by the following characteristics: coagulation of rabbit plasma at 4 h (26 versus 100%, respectively), hemolysis of sheep blood at 24 h (30 versus 79%, respectively), and mannitol fermentation at 24 h (4 versus 93%, respectively). A clear separation of the two species was apparent only with the acetoin (modified Voges-Proskauer) reaction (100% of the *S. aureus* isolates versus 0% of the *S. intermedius* isolates) and β-galactosidase activity on the API Staph-Ident strip (0% of the *S. aureus* isolates and 100% of the *S. intermedius* isolates). Susceptibilities of *S. intermedius* and *S. aureus* were 72 and 7%, respectively, to penicillin G, and 100% of both species to oxacillin. Fourteen previously collected strains of coagulase-positive staphylococci from infected canine-inflicted human wounds were reanalyzed; 3 of 14 (21%) isolates were *S. intermedius*. We conclude that *S. intermedius* is a common canine gingival flora and is responsible for some canine-inflicted human wound infections, thus representing a newly recognized zoonotic pathogen.

It is estimated that more than 1.5 million humans are bitten by dogs each year and that 5 to 29% of the wounds become infected (9, 24, 28, 36, 38). Series of canine-inflicted bite wounds in humans indicate that one of the predominant pathogens is *Staphylococcus aureus*, accounting for 10 to 50% of bacterial isolates (9, 10, 15, 16, 18, 19, 31).

In 1976, Hajek identified a separate coagulase-positive staphylococcal species (20). This organism, *S. intermedius*, is clearly distinguishable from *S. aureus* by its microbiological and biochemical characteristics. *S. intermedius* has since been identified in dogs, mink, horses, and cats as common flora (2, 11, 14, 17, 20). The organism has also been shown to be an invasive pathogen causing skin, urinary tract, bone, and central nervous system infections in several nonhuman animal species (7, 22, 27, 32, 33). Because clinical bacteriology laboratories may misidentify *S. intermedius* as *S. aureus*, we reexamined the microbiological characteristics, canine carriage rates, and antibiotic susceptibilities of *S. intermedius* and *S. aureus* isolated from gingival cultures of the upper front teeth of 135 healthy dogs. In addition, we document three cases of *S. intermedius* isolated from clinically infected dog bite wounds in humans.

MATERIALS AND METHODS

Subjects. A total of 135 consecutive healthy dogs seen at an urban veterinary clinic (Pet Medical Clinic, Santa Monica, Calif.) by a licensed veterinarian (A. Staatz) from March to June 1987 were studied. The dogs were seen for routine physicals, vaccinations, and preoperative exams for neuters. Dogs were excluded if they were less than 1 year of age, had a chronic illness, had evidence of infection, or had been on antibiotics within 7 days of evaluation. The age, sex, breed, and size of each dog were recorded. Working breeds were defined as shepherds, retrievers, and huskies. The predominant habitat of each animal (indoor or outdoor) and the histories of prior antibiotic administration and of steroid therapy were also noted.

Isolation and identification procedures. The gingival surface of the upper front teeth of each dog was swabbed with a sterile cotton-tipped applicator. Specimens were immediately streaked onto mannitol-salt agar (Difco Laboratories, Detroit, Mich.), and cultures were incubated for 48 h at 37°C.

All colony types from primary cultures were Gram stained and tested for catalase activity. Colonies showing mannitol fermentation within 48 h were plated onto bile esculin azide agar (Difco). Gram-positive, catalase-positive, and bile esculin-negative isolates were tested further.

Mannitol fermentation was determined on mannitol-salt plates at 24 and 48 h. Mannose fermentation was completed on 1% mannose in phenol red broth base (Difco). Hemolysis was determined on tryptic soy agar II–5% sheep blood agar (Scott Laboratories, Inc., Fiskeville, R.I.) at 24 and 48 h. Coagulase production was determined by the tube coagulase test using reconstituted sodium citrate rabbit plasma (BBL Microbiology Systems, Cockeysville, Md.). Readings were made after 4 h of incubation at 37.0°C and after 24 h at room temperature. Solid clots at 4 h were considered positive, gelatinous or solid clots at 24 h were considered delayed,

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TABLE 1. Characteristics of dogs with *S. intermedius* and *S. aureus* isolated from gingival surfaces[a]

Isolate	Age (yr; mean ± SD)	Sex ratio (M/F[b])	% of dogs >40 lb (ca. 18 kg)	% of dogs of working breeds	% of dogs with history of:		% of dogs with habitat		
					Steroid therapy	Prior antibiotic treatment	Indoor	Outdoor	Both
S. intermedius (n = 53)	6.6 ± 4.0	0.7	61	48	15	78	59	18	23
S. aureus (n = 14)	6.4 ± 4.3	1.8	100	100	35	81	0	29	71

[a] $P < 0.05$ for figures on weight, breed, and indoor and both habitats. P not significant for other data.
[b] M/F, Male/female.

partial or fibrous clots at 24 h were considered weakly positive, and no clot was considered negative.

Acetoin production was tested by a modification of the method of Barritt (6). MR-VP broth (0.5 ml; Difco Laboratories, Detroit, Mich.) and one colony of bacteria were incubated in a sterile tube (100 by 13 mm) for 18 to 24 h at 37.0°C. Following incubation, 3 drops of 5% α-naphthol in absolute alcohol and 2 drops of 40% potassium hydroxide were added. After 15 min, the tube was observed for a pink-to-red color which did not fade (positive reaction).

In addition, isolates were subjected to the species identification system by API Staph-Ident (Analytab Products, Plainview, N.Y.), which uses a battery of 10 microbiochemical tests including alkaline phosphatase, urease, β-glucosidase, β-glucuronidase, β-galactosidase, and aerobic acid formation from D(+)-mannose, D-mannitol, D-(+)-trehalose, and salicin, and utilization of arginine. Staph-Ident strips were inoculated, incubated, read, and scored according to the instructions of the manufacturer. Identifications were obtained from the Staph-Ident profile register.

The following standard strains were used in all experiments to provide verification of standard isolation procedures and the results of biochemical testing: *S. aureus* ATCC 29213 and 25923; *S. intermedius* ATCC 29663; *S. sciuri* ATCC 29060; *S. simulans* ATCC 27851; and *S. epidermidis* ATCC 14990.

Antimicrobial susceptibility determinations. All *S. intermedius* and *S. aureus* isolates were tested according to National Committee for Clinical Laboratory Standards disk susceptibility methods on Mueller-Hinton agar plates (BBL) for susceptibilities to the following antibiotics: amoxicillin-clavulanic acid, chloramphenicol, clindamycin, cephalothin, ceftriazone, cefotaxime, erythromycin, gentamicin, oxacillin, penicillin, ampicillin-sulbactam, and vancomycin (29).

Canine-inflicted human wound infection isolates. A total of 14 isolates from canine-inflicted human wound infections were obtained (E. Goldstein). These isolates had been identified as *S. aureus* previously and were subjected to staphylococcal identification procedures as outlined above (19).

Statistical analysis. Data were analyzed by using the chi-square test. Statistical significance was defined by $P < 0.05$.

RESULTS

Gingival cultures were taken from each of 135 dogs, 48% male and 52% female. The mean age (± standard deviation) of the dogs was 5.8 ± 3.8 years, with a range of 1 to 16 years. Table 1 shows the characteristics of dogs from which *S. intermedius* or *S. aureus* was isolated. There was no significant difference in regard to the age of dogs or prior antibiotic or steroid exposure in those with *S. aureus* or *S. intermedius*. *S. intermedius* was found more frequently in female dogs than was *S. aureus* (P = 0.14). Dogs with *S. intermedius* were smaller than dogs with *S. aureus* ($P < 0.05$), and fewer dogs with *S. intermedius* were of working breeds compared with dogs with *S. aureus* ($P < 0.05$). Dogs with *S. intermedius* lived predominantly in indoor habitats compared with dogs with *S. aureus* ($P < 0.05$).

Microbiological characteristics. The microbiological characteristics of the 53 *S. intermedius* and 14 *S. aureus* isolates are shown in Table 2; these isolates were cultured from 39 and 10% of the dogs, respectively. *S. intermedius* and *S. aureus* were isolated concomitantly in only three (2%) dogs. Coagulase reactions were positive after incubation for 24 h for all *S. intermedius* and *S. aureus* isolates; the tests were positive after 4 h for 26% of the *S. intermedius* isolates, compared with 100% of the *S. aureus* isolates. Delayed hemolysis, mannitol fermentation, and coagulation were generally characteristic of *S. intermedius*. However, a clear separation of the two species was possible only with the acetoin (modified Voges-Proskauer) reaction: 0% of the *S. intermedius* isolates were reactive, compared with 100% of the *S. aureus* isolates.

The biochemical characteristics of *S. intermedius* and *S. aureus* isolates as determined by the API Staph-Ident system are listed in Table 3. A clear separation of *S. intermedius* and *S. aureus* was possible only with the β-galactosidase reaction; 100% of the *S. intermedius* isolates were positive, versus 0% of the *S. aureus* isolates. Both mannitol and mannose fermentation API reactions were difficult to read or subject to interobserver error; thus, these results were not consistent with standard reactions observed on 1% mannitol and 1% mannose agars.

Antibiotic susceptibilities. The antibiotic susceptibilities of *S. intermedius* and *S. aureus* isolates are presented in Table 4. *S. intermedius* was susceptible to a wide range of antibiotics with the exception of penicillin, to which 28% of the isolates were resistant. *S. aureus* had an antibiotic susceptibility pattern similar to that of *S. intermedius* except 93% of the *S. aureus* isolates were resistant to penicillin. There was no relationship between prior exposure to antibiotics and antibiotic resistance for dogs with *S. intermedius* or *S. aureus*.

Canine-inflicted human wound isolates. Of 14 isolates from clinically infected canine-inflicted human wounds which were previously identified as *S. aureus*, 3 (21%) were reclas-

TABLE 2. Microbiological profiles of *S. intermedius* and *S. aureus* isolated from canine gingival surfaces

Isolate	% Positive for:							
	Pigment	Mannitol[a]		Hemolysis		Coagulase		Acetoin
		24 h	48 h	24 h	48 h	4 h	24 h	
S. intermedius (n = 53)	0	4	91	30	91	26	100	0
S. aureus (n = 14)	71	93	100	79	100	100	100	100

[a] Mannitol fermentation.

TABLE 3. Biochemical reactions of *S. intermedius* and *S. aureus* on API Staph-Ident system

Isolate	% Positive on substrate[a]									
	PHS	URE	GLS	MNE	MAN	TRE	SAL	GLC	ARG	NGP
S. intermedius (n = 53)	100	100	40	58	0	90	0	0	98	100
S. aureus (n = 14)	64	78	64	64	43	93	0	0	100	0

[a] Abbreviations: PHS, phosphatase; URE, urease; GLS, β-glucosidase; MNE, mannose; MAN, mannitol; TRE, trehalose; SAL, salicin; GLC, β-glucuronidase; ARG, arginine dihydrolase; NGP, β-galactosidase.

sified as *S. intermedius*. All three isolates were nonpigmented, catalase and coagulase positive (delayed), acetoin negative, and API Staph-Ident β-galactosidase positive. Two of these isolates were susceptible and one was resistant to penicillin; all were susceptible to amoxicillin-clavulanic acid, chloramphenicol, clindamycin, cephalothin, ceftriaxone, cefotaxime, erythromycin, gentamicin, oxacillin, ampicillin-sulbactam, and vancomycin.

DISCUSSION

Coagulase-positive staphylococci have been recognized as nasal-oral floras in 3 to 72% of various animals (1, 4, 8, 23, 34, 35). In 1976, Hajek described *S. intermedius*, a coagulase-positive staphylococcus, previously classified as *S. aureus* biotypes E and F, which differed from other *S. aureus* in biochemical reactions and in cell wall composition (20, 30). *S. intermedius* subsequently has been found as nasal flora in dogs, pigeons, mink, cats, and horses and has been identified as a pathogen in a variety of animals with skin, urinary, respiratory, bone, reproductive tract, and central nervous system infections (2, 7, 11, 14, 17, 20, 22, 27, 32, 33). *S. intermedius* is the predominant cause of staphylococcal skin infections among dogs (7, 27). Studies of the antibiotic susceptibilities of coagulase-positive staphylococci in general, and *S. intermedius* and *S. aureus* specifically, from animal sources have revealed inconsistent findings, particularly with regard to susceptibility to penicillin (7, 12, 21, 22, 26, 27, 37).

While *S. intermedius* has been clearly established as a flora and a pathogen among animals, to our knowledge this organism has never been identified from human specimens. Series of dog bites to humans have revealed *S. aureus* to be associated with 10 to 50% of canine-inflicted wounds (9, 10, 15, 16, 18, 19, 31). Most series have shown these staphylococcal isolates to be resistant to penicillin (19); however, some studies have noted penicillin-susceptible isolates (31), including one report in which 3 of 4 isolates were susceptible to penicillin (10). Since human clinical bacteriology labs do not attempt to further differentiate coagulase-positive staphylococcal species and since *S. intermedius* is clearly a skin pathogen in animals, this staphylococcal organism may be a human pathogen in canine-inflicted wounds. Our three cases of *S. intermedius* associated with dog bite infections support this concept.

Of 135 dogs studied, *S. intermedius* was more commonly found to be a gingival flora of the upper front teeth than *S. aureus*. However, *S. aureus* was more likely to be found in animals that are prone to bite (13, 24); that is, *S. aureus* was more common among males, large dogs, and those of working breeds than was *S. intermedius*. *S. intermedius* and *S. aureus* were cultured together in only 2% of the animals, suggesting that the organisms may be mutually exclusive canine floras. Balusek and Hajek have reported that 2% of *S. aureus* and 18% of *S. intermedius* strains demonstrate inhibition of the growth of the other species ("staphylococcin effect") (5).

S. intermedius was found significantly more often among dogs of indoor habitats than *S. aureus*. This phenomenon may in part be explained by differences in the degrees of exposure of these dogs to other animals and human beings, as this has been shown to affect the carriage rates of these staphylococcal species among dogs and cats (3, 11, 35).

The antibiotic susceptibility patterns of *S. intermedius* and *S. aureus* were similar except that the former was more susceptible to penicillin, 72 versus 7%, respectively. *S. intermedius* is more often susceptible to penicillin, and this may explain the findings of previous studies which have revealed a high incidence of penicillin-susceptible coagulase-positive staphylococci from canine specimens (4, 21, 26, 37) and isolates from human dog bite wounds (10, 31). Other studies of *S. intermedius* isolated from canine infections have demonstrated more resistance to penicillin than was found in our study, with 50% (12), 39% (7), and 17% (27) of isolates susceptible, and in one study, β-lactamase was found in 55% of *S. intermedius* isolates (22). We were unable to find a significant relationship between resistance to penicillin and prior exposure to this or other antibiotics.

With regard to the identification of *S. intermedius*, our findings are consistent with the original findings of Hajek and others (14, 20, 33). All of the isolates were nonpigmented, catalase and coagulase positive, and acetoin negative. Only acetoin production clearly separated *S. intermedius* and *S. aureus*. The API Staph-Ident system confirmed our initial identification of *S. intermedius* and *S. aureus* by standard microbiological methods for almost all isolates, including those isolates from human wounds. Of the 10 API biochemical tests, only β-galactosidase activity clearly distinguished *S. intermedius* and *S. aureus*; this is consistent with previous

TABLE 4. Antibiotic susceptibilities of *S. intermedius* and *S. aureus*

Isolate	% Susceptible to[a]:											
	Amc	C	CC	CF	Cro	Ctx	E	Gm	Ox	P	Sam	Va
S. intermedius (n = 53)	100	98	98	98	100	100	96	100	100	72	100	100
S. aureus (n =14)	100	100	100	100	100	100	100	100	100	7	100	100

[a] Abbreviations: Amc, amoxicillin-clavulanic acid; C, chloramphenicol; CC, clindamycin; CF, cephalothin; Cro, ceftriaxone; Ctx, cefotaxime; E, erythromycin; Gm, gentamicin; Ox, oxacillin; P, penicillin; Sam, ampicillin-sulbactam; Va, vancomycin.

reports (25). Both API Staph-Ident strip and mannitol-salt agar results indicated that mannitol fermentation is an unreliable discriminator of *S. intermedius* and *S. aureus*. In summary, all *S. intermedius* isolates were acetoin negative and β-galactosidase positive, and all *S. aureus* isolates were acetoin positive and β-galactosidase negative.

To our knowledge, the three isolates of *S. intermedius* from dog bite wounds described in this report are the first such cases to be associated with human infection. One previous study reported the isolation of *S. intermedius* from an abscess in a human being (2). However, the identification of this isolate as *S. intermedius* is highly questionable because of the presence of a positive acetoin reaction and maltose fermentation. Another previous report which demonstrated enterotoxin-producing stains in 43% of dogs with *S. intermedius* suggested that this organism could have a pathogenic role in cases of human food poisoning (17), but this clinical possibility has never been confirmed. It appears that *S. intermedius* may be a relatively common and potentially invasive pathogen of canine-inflicted human wounds. Clinical laboratories should consider differentiating coagulase-positive staphylococci isolated from canine-inflicted wounds.

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