Species Distribution of Coagulase-Positive Staphylococci in Animals

ERNST L. BIBERSTEIN,* SPENCER S. JANG, AND DWIGHT C. HIRSH

Microbiology Service, Veterinary Medical Teaching Hospital, University of California, Davis, California 95616

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A total of 268 isolates of coagulase-positive staphylococci from a variety of animal species, including dogs, horses, cats, monkeys, goats, and cows, were assigned to species on the basis of the API Staph-Ident system (Analytab Products, Inc., Plainview, N.Y.). Of 195 isolates from dogs, 179 (91.8%) were *Staphylococcus intermedius*, as were 9 of 25 (36%) isolates from horses, 7 of 15 (46.6%) isolates from cats, and 4 of 6 (66.6%) isolates from goats. Only 1 of 10 isolates from monkeys and none of 7 isolates from cows were *S. intermedius*. Of the remaining 68 cultures, 63 were identified as *Staphylococcus aureus* and 5 as *Staphylococcus hyicus*. The latter identifications were rendered doubtful on the basis of conventional tests. Identification appeared to be more certain in the *S. aureus* sample than in the *S. intermedius* sample. Distribution of biotypes within the two bacterial species as represented by different API profile numbers and reactivity on test substrates showed no significant variations among the host species, except for the *S. aureus* biotypes in dogs. Both *Staphylococcus* species were represented about equally among samples from different tissues and lesions, apart from skin-related infections in dogs, which were associated exclusively with *S. intermedius* (P < 0.01). Differences between *S. aureus* and *S. intermedius* in antimicrobial susceptibility patterns, prevalence of clumping factor, and occurrence of beta-toxin were found to be not significant.

Until relatively recently, all coagulase-positive staphylococci, with the possible exception of some *Staphylococcus hyicus* strains originating from typical exudative epidermitis in swine, were identified as *Staphylococcus aureus* (2). The taxonomic upheaval to which the genus has been subjected during the last decade, however, has resulted in the establishment of three species made up, either entirely or in part, of coagulase-positive strains, viz., *S. aureus*, *Staphylococcus intermedius*, and *S. hyicus* (6, 7). In view of this new development, an examination of clinical isolates of diverse animal origin with regard to the distribution of the three newly described species appeared desirable.

This was the primary object of the present study. The validation, by a recent investigation (13), of the API Staph-Ident system (Analytab Products, Inc., Plainview, N.Y.) as a rapid and accurate method for the identification of staphy-lococcal species pointed to a convenient way to carry out this study in the course of routine diagnostic operations (7). An ulterior purpose was to determine whether the precise species identification of coagulase-positive staphylococci, a procedure involving additional expense in time, labor, or material, was necessary or even justified from the point of view of useful information generated for the guidance of clinicians.

MATERIALS AND METHODS

Cultures. All coagulase-positive staphylococcal strains isolated at the Microbiology Service, Veterinary Medical Teaching Hospital, University of California, Davis, between July 1982 and July 1983 were subjected to species identification procedures by the Staph-Ident system. A total of 268 isolates were examined. The details of their zoological and anatomical-pathological derivation are given in Tables 1 and 2. All isolations were carried out on bovine blood agar (5%). Hemolytic reactions were noted, particularly with respect to beta-toxin activity manifested by the typical hot-cold lysis sequence.

Coagulase tests. All catalase-positive, gram-positive cocci were tested for clumping factor (slide coagulase) in rabbit plasma (coagulase plasma EDTA; Difco Laboratories, Detroit, Mich.) (4). Observation time was up to 30 s. All strains giving a negative reaction were tested by the tube method (4) with 0.5 ml each of plasma and an overnight broth culture in brain heart infusion broth. Incubation was carried out for 4 h at 37° C and was extended to overnight for strains giving negative results at 4 h.

Species identification. 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. In a few cases (see Results) in which coagulase-positive strains were identified as coagulase-negative species, the evidence of source and hemolytic and coagulase activities were accepted as overriding the API first choice and led to the selection of a more remote choice consistent with such evidence. Significance of differences in distribution of staphylococcal species and biotypes among various hosts and pathological-anatomical sources was analyzed by the Yates chi-square or the Fisher test (14).

Antimicrobial susceptibility determinations. A total of 193 of the cultures were tested for susceptibility to some or all of the following antimicrobial agents: penicillin, ampicillin, oxacillin, carbenicillin, cephalothin, erythromycin, chloramphenicol, tetracycline, gentamicin, kanamycin, amikacin, and trimethoprim-sulfamethoxazole. All but three of the strains were tested by a microdilution procedure (MIC 2000; Dynatech Laboratories, Inc., Alexandria, Va.) in which graded amounts of antimicrobial agents were employed in Mueller-Hinton broth in a microdilution plate (8 by 12 wells) and which was aimed at determining a MIC. The three exceptions were tested by a modification of the Kirby-Bauer method (3). Since the majority of the strains were either susceptible to less than the minimum amount provided or resistant to more than the maximum amount of some drugs, no valid means and standard deviations could be calculated for comparison of species susceptibilities. Instead, strains were classified as susceptible to a particular drug if the MIC

^{*} Corresponding author.

fell below the following values (in micrograms per milliliter): penicillin, 0.1; oxacillin, 2; carbenicillin, 64; cephalothin, 8; erythromycin, 2; chloramphenicol, 8; tetracycline, 4; gentamicin, 4; kanamycin, 8; amikacin, 16; trimethoprim-sulfamethoxazole, 2-38; and resistant if it exceeded these concentrations. Tests for penicillinase were done on isolates with a penicillin MIC of $\leq 0.1 \ \mu$ g/ml (Marion Scientific, Kansas City, Mo.). Significance of differences in susceptibilities was tested by Yates chi-square test (10).

RESULTS

The distribution of S. aureus and S. intermedius among the various animal species and sample types is shown in Table 1. Of the 268 isolates examined, 63 were identified as S. aureus and 200 as S. intermedius. One of the S. aureus strains and 10 of the S. intermedius strains were identified as such by the Staph-Ident system only by the second or even the third choice (Table 2). The preponderance of S. intermedius isolates in the total sample is clearly referable to the prominence of dogs (195 of 268; 72.8%) among our sample sources. In this host, S. intermedius predominated by a ratio of more than 11:1. This distribution differed significantly (P < 0.002) from that in horses, cats, monkeys, and cattle, in which S. aureus was more common. Of the 268 coagulasepositive staphylococci, 5 were identified by the Staph-Ident system as S. hyicus (Table 1). Two of these strains were recovered from dogs with otitis externa and urinary tract infections, respectively. Of three equine isolates, one came from an infected surgical incision, another from pus, and the third from granulation tissue. Four of the five strains produced beta-toxin (hemolysin), and all produced clumping factor, neither of which is characteristic of S. hyicus (5). Since, moreover, isolation of authentic strains of S. hyicus from dogs has, to our knowledge, not been documented, we have some reservations concerning the reliability of the identifications. The profile numbers involved, 7540 and 5540, are accompanied in the index by the note: "check source—if man = aureus." In the absence of instructions regarding animal strains, we have disregarded these five strains in our analysis.

Efforts were made to discover whether the two staphylococcal species, in addition to exhibiting different host predilections, also showed different distribution patterns with regard to their tissue or pathogenic specificities. Such differences were substantiated only in the case of isolates recovered from dogs with skin and wound infections, in which S. intermedius was found to the exclusion of S. aureus (P <0.0l).

Within each species, a spectrum of different reaction patterns with respect to the 10 substrates provided in the API gallery was registered (see Tables 2 and 3). In general, our observations, with some exceptions noted below, agreed with those of other workers (Table 3). We attempted to relate biotypes to host species and sample type. In case of S. aureus biotype 7740, i.e., positive on all substrates except salicin, β-glucuronidase, and 2-naphthol-β-D-galactopyranoside, a significant difference in distribution among the several host species was noted. This biotype was with 29 representatives, the predominant biotype among the 63 strains of S. aureus (46%), but in dogs it represented only 3 of 16 (18.8%); P < 0.05). Comparison between canine and equine strains also revealed a significant relative preponderance of biotype 7740 among the latter (P < 0.05). This discrepancy between canine and other S. aureus strains was not due to a particular identifiable reaction in the API battery. It was noted, however, that 2-naphthol-β-D-galacto-

E 1. Coagulase-positive staphylococcal isolates from anii No. (%) of strains from follow No. (%) of strains from follow Dogs (n = 15) Cats (n = 25) SI SA SI SI SA SI 40 (22.3) 1 (12.5) 25 (13.9) 5 (31.3) 3 (33.3) 1 (12.5) 25 (13.9) 5 (31.3) 3 (33.3) 1 (12.5) 1 (14.3) 2 (0) 10 (5.6) 3 (18.8) 2 (0)	mals ($n =$ ving source ² Monkeys ($n = 11$) SA SI SA SI 20.0) 1		= loat	SI
40 (22.3) 25 (13.9) 10 (5.6) 10 (5.6)).0) 1			
Skeletal system ^e	0.0)			1
Keproductive tract (including mammary glands)).0)	2		دى
Total % 8.2 91.8 64.0 36.0 53.3 46.6 90.0		10.0 33.3 66.3	:3 6	56.7 100

TABLE	2.	Staphylococcal	profiles repres	ented by mor	e than one strain

Species and	No. of strains from following source:									
biotype	Dogs	Horses	Cats	Monkeys	Cattle	Goats	Other	Total %		
S. aureus ^a										
7740	3	10	5	6	3	0	2	46		
5740	3	3	1	0	2	1	0	15.9		
7700	3	0	0	1	0	0	0	6.3		
5700	0	1	0	1	1	1	0	6.3		
5741 ^b	3	0	0	0	0	0	0	4.8		
3740	2	0	0	1	0	0	0	4.8		
7340	0	0	0	0	0	0	2	3.2		
5340	0	2	0	0	0	0	0	3.2		
S. intermedius ^c										
7541	112	6	4	0	0	0	0	61.0		
3541	19	0	2	1	0	0	0	11.0		
7741	5	3	0	0	0	4	Ō	6.0		
7441	11	0	0	0	0	0	Ō	5.5		
7041	9	0	0	0	Õ	õ	Õ	4.5		
7501	4	0	0	0	Ō	õ	Õ	2.0		
3141	3	0	Ő	0	Ō	õ	Ŏ	1.5		
3041 ^d	3	Ō	Ō	Ő	Õ	Ő	Ő	1.5		
3441 ^d	2	Ō	Ō	Ő	Õ	Ő	Ő	1.1		
3501	2	Ō	Ō	Ő	õ	Ő	Ő	1.0		
5541	2	Õ	Ŏ	Õ	ŏ	ŏ	ŏ	1.0		

^a Profiles represented by one strain were 1700, 3700, 5500, 6701 (identified as S. xylosus by the Staph-Identsystem; S. aureus was second choice [see Discussion]), 7500, and 7710 (total, 9.7%).

^b See Discussion.

^c Profiles represented by one strain were 3101; 3401 and 3701 (see footnote *d*, below); 5141; 7141; and 7401, 7531, and 7641 (identified as *S*. *xylosus* by the Staph-Identsystem; *S*. *intermedius* was second choice for 7641 and 7531 and third choice [after *S*. *saprophyticus*] for 7401) (total, 4.0%).

^d Identified as S. simulans by the Staph-Identsystem; S. intermedius was second choice for 3441 and 3701 third choice (after S. saprophyticus) for 3401 and 5141, and third choice after (S. epidermidis) for 3041.

sidase activity, which has not been observed in S. aureus, was peculiar to the canine sample (P < 0.01). It was further observed that arginine dihydrolase activity was a great deal more common among our S. aureus cultures (regardless of origin) than those forming the data base of the API figures (P << 0.002).

No unusual distribution or reactivity patterns were observed among the *S. intermedius* isolates.

The reactivity of the isolates in the test for clumping factor (slide coagulase) is shown in Table 4. No significant differences (P > 0.05) in the occurrence of clumping factor among the species of our sample were observable: 57 of 63 (90.5%)

S. aureus isolates and 168 of 200 (84%) S. intermedius isolates gave positive test results. All of five strains identified as S. hyicus by the Staph-Ident system possessed clumping factor.

Hemolytic activity was apparent with all but 7 of the 268 strains (97.4%) (Table 5). The predominant pattern was that associated with beta-toxin (phospholipase C), regardless of the staphylococcal species involved: 126 of 200 (63%) S. *intermedius* isolates and 36 of 63 (60.3%) S. *aureus* isolates showed beta-toxin activity. The difference between the two species in this regard was not significant (P > 0.10), nor were the differences in distribution of hemolytic pattern between

TABLE 3. Biochemical reactions of coagulase-positive staphylococci on API substrates

				% Pos	itive on follo	owing substr	rate ^a :			
Strain and data source	PHS	URE	GLS	MNE	MAN	TRE	SAL	GLC	ARG	NGP
S. aureus										
Kloos and Wolfshohl (13) $(n = 20)$	100	60-70	80	100	90	100	0	0	80-85	0
API data base (1) $(n = \text{unknown})$	60	80	97	100	99	90	0	0	10*	0
Present study										
All S. aureus $(n = 63)$	98.4	68.7	92.1	100	96.8	92.1	1.6	0	77.7	6.3
All but dogs $(n = 47)$	100	68.1	95.7	100	97.9	89.4	0	Ō	80.9	0
Dogs only $(n = 16)$	93.8	62.5	87.5	100	100	100	6.3	0	68.8	25*
S. intermedius										
Kloos and Wolfshohl (9) $(n = 20)$	100	100	55-65	100	0	100	0	0	85-90	100
API data base $(n = \text{unknown})$	100	100	60	100	ŏ	90	õ	Õ	93	100
Present study $(n = 200)$	100	98.5	82.5	85	7.5	91	0.5	0.5	94.5	100

^{*a*} Abbreviations: PHS, phosphatase; URE, urease; GLS, β -glucosidase; MNE, mannose; MAN, mannitol; TRE, trehalose; SAL, salicin; GLC, β -glucuronidase; ARG, arginine dihydrolase; NGP, 2-naphthol- β -D-galactopyranosidase. Significantly different from rest of sample (P < 0.01). See Discussion.

Species and host	Clumping factor strain	
-	Present	Absent
S. aureus		
Dog	13 (81.25)	3
Horse	15 (93.75)	1
Cat	8 (100)	0
Monkey	9 (90)	1
Cattle		0
Goat	2 (100)	0
Other	3 (75)	1
Total %	90.5	
S. intermedius		
Dog	149 (83.74)	30
Horse		2
Cat	5 (71.43)	2
Monkey	1 (100)	0
Cattle	0	0
Goat	4 (100)	0
Other	0	0
Total %	84.0	

TABLE 4. Clumping factor in S. aureus and S. intermedius by host species

strains from different host species (P > 0.05). The observation of beta-toxin activity among four of five isolates identified by the Staph-Ident system as S. hyicus has been mentioned before.

Antimicrobial susceptibilities of S. aureus and S. intermedius strains are shown in Table 6. None of the differences observed could be shown to be statistically significant. Not shown in Table 6 is the comparative prevalence of penicillinase producers among strains requiring an MIC of ≤ 0.1 µg/ml. This was 1 of 7 for S. aureus and 3 of 30 for S. intermedius (P > 0.05).

TABLE 5. Hemolytic patterns of staphylococci from animals

Species and host		strains exhibiti ng hemolysis:	ing follow-
- -	Beta-toxin	Other	None
S. aureus			
Dog	10 (62.5)	6 (37.5)	0
Horse	8 (50.0)	7 (43.8)	1 (6.2)
Cat	4 (50.0)	3 (37.5)	1 (2.5)
Monkey	9 (90.0)	1 (10.0)	0
Cattle	5 (71.4)	2 (28.6)	0
Goat	1 (50.0)	1 (50.0)	0
Other	1 (25.5)	2 (50.0)	1 (25.0)
Total %	60.3	34.9	4.8
S. intermedius			
Dog	119 (66.1)	57 (31.7)	4 (2.2)
Horse	4 (44.4)	5 (55.6)	0
Cat	2 (33.3)	4 (60.7)	0
Monkey	0	1 (100)	0
Cattle	0	0	0
Goat	1 (25.5)	3 (75.0)	0
Other	0	0	0
Total %	63.0	35.0	2.0

DISCUSSION

The representation of the various host species in our sample is a reflection more of the composition of the patient population at this hospital than of the relative prevalence of staphylococcal infections among these species: although dogs are commonly subject to such infections, so are dairy cattle. Our figures, therefore, reveal distributions within but not among species.

Previous reports had documented the occurrence of S. intermedius in dogs, horses, pigeons, mink, foxes, raccoons, and gray squirrels (8-12, 15). All of the studies cited dealt with commensal strains from normal animals. A later investigation by Phillips and Kloos included five canine strains, four of which originated from animals with pyoderma (16). In a subsequent personal communication, Phillips stated that over 90% of an unspecified number of coagulase-positive staphylococcal isolates from dogs with pyoderma were S. intermedius. In our sample, all of 73 strains originating from epidermal sources, including pyoderma, wound, and external ear infections, were S. intermedius. This group differed significantly (P < 0.01) from other canine staphylococcal infections, a proportion of which (12.12%) were associated with S. aureus. We would conclude that canine skin infections, and particularly pyoderma, long known to involve coagulase-positive staphylococci as the only significant bacterial component, are in our practice area associated essentially with S. intermedius only, whereas staphylococci from other canine sources have a 10 to 15% chance of being S. aureus.

Unlike Hájek, we found among 25 equine isolates about 2 of 3 (64%) to be S. aureus. The difference is possibly due to the clinical nature of our sources, whereas Hájek worked with nasal isolates of normal animals. In 15 feline specimens, the two species were about evenly divided, whereas in monkeys and cattle, S. aureus was clearly the predominant species. All differences in distribution of the two staphylococcal species among the various host species (other than dogs) lacked statistical significance, probably owing in part to the small numbers of strains. Likewise, the significance of any variations in the occurrence of S. aureus and S. intermedius in tissues and lesions of the diverse host species could not be statistically confirmed.

The representation of different biotypes among S. aureus strains showed some deviation from that observed on human strains by Kloos and Wolfshohl (13), especially when host sources are considered. The leading biotype identified by these authors, 7740, accounting for over half of their strains, was also the most frequent one in our animal sample as a whole (46%), but represented less than 20% of the canine isolates. Although this difference is not significant (P > 0.5), that between the canine and the other animal strains with respect to this profile is (P < 0.5). Of the eight biotypes (7740, 5740, 3700, 1740, 1700, 7540, 5700, and 3740) represented among the 20 strains of Kloos and Wolfshohl, six (7740, 5740, 3700, 1700, 5700, and 3740) are also present among the 63 animal strains, and three (7740, 5740, ard 3740) among the canine isolates, comprising seven biotypes.

When considering individual biochemical reactions of isolates identified as *S. aureus*, we found that the prevalence of arginine dihydrolase activity was much greater in our sample (P << 0.002) than indicated in the API data (1) and approximates that observed by Kloos and Wolfshohl (13). Only canine strains showed 2-naphthol- β -D-galactopyranosidase activity (P < 0.01). It has since been suggested to us that the four strains involved may in fact have been mannitol-fermenting variants of *S. intermedius*. Since the cultures

TABLE 6. Antimicrobial susceptibilities of coagulase-positive staphylococci from animals

	S	6. aureus	S. i	ntermedius	
Antimicrobial agent	Total	No. (%) resistant	Total	No. (%) resistant	Р
Penicillin	. 26	20 (70)	124	76 (61.3)	≫0.1
Oxacillin	• •	1 (4.4)	124	4 (3.2)	$\gg 0.1$
Carbenicillin	. 24	0 (0)	117	1 (0.9)	$\gg 0.1$
Cephalothin		0 (0)	124	1 (0.8)	$\gg 0.1$
Erythromycin	. 25	1 (4.2)	124	13 (10.5)	>0.1
Chloramphenicol	. 30	4 (15.4)	163	16 (9.8)	$\gg 0.1$
Tetracycline	. 30	7 (23.3)	163	65 (39.9)	>0.1
Gentamicin	. 30	1 (3.5)	163	2 (1.2)	$\gg 0.1$
Kanamycin		2 (7.0)	158	12 (7.6)	$\gg 0.1$
Amikacin	. 22	0 (0)	154	1 (0.7)	$\gg 0.1$
Trimethoprim-sulfamethoxazole		0 (0)	161	6 (3.7)	≫0.1

were no longer available for either reexamination or additional testing, this question could not be addressed directly. The following considerations, however, are offered in support of the Staph-Ident identifications: one of the four strains, biotype 6701, was negative on the arginine utilization test, but fermented mannitol. Among our 200 S. intermedius isolates, none exhibited a combination of these traits, whereas of the 59 undisputed S. aureus cultures, 12 (20.3%) did. The API listing, which offers four possible species under this profile number as increasingly improbable choices, does not include S. intermedius as even a remote possibility. The other 3 strains positive for 2-naphthol-B-D-galactopyranosidase, identified as S. aureus under profile number 5741 ("good identification"), showed a combination of a negative urease with a positive mannitol reaction, which was not seen among our 200 S. intermedius cultures, but was quite common (30.5%) among the 59 S. aureus strains negative for 2-naphthol-β-D-galactopyranosidase. Neither the API data (1) nor the Kloos and Wolfshohl study (13) record negative urease or positive mannitol reactions, let alone a combination of the two, for any S. intermedius strain. We are therefore inclined to consider as quite remote the possibility that these four cultures are S. intermedius and to accept the Staph-Ident identification, particularly in view of the already established distinctness of biotype distribution among canine strains of S. aureus. Although, in view of the small number of canine S. aureus strains in our sample and of S. aureus strains in the study of Kloos and Wolfshohl, the significance of most of these apparent peculiarities of dog-derived cultures could not always be statistically confirmed, the suggestion that canine S. aureus strains represent a special population is strong enough to deserve further investigation.

With S. intermedius, the two profile types accounting for over two-thirds of our cultures (7541 and 3541) are also those describing the bulk (65 to 90%) of the collection of Kloos and Wolfshohl (13). The only difference between our and their observations was a higher prevalence (P. < 0.05) of β -glucosidase activity among our strains.

It is worthy of note, however, that among our 200 S. *intermedius* strains, the identification of 85 (42.5%) was characterized as less than "excellent" by the API, as contrasted to only 15 (23.8%) of the S. *aureus* strains (P < 0.05). Ten strains, in fact, were identified by the index as something other than S. *intermedius* (Table 2), and only consideration of the positive coagulase test made this species the only one possible among the choices offered. We suspect that the identification profiles for S. *intermedius* are derived from a much less extensive data base than those for

S. aureus and that the full extent and frequency of variant reactivity spectra in the former species remain to be firmly defined.

The data of Hájek presented in support of his proposal for establishment of the species S. *intermedius* suggest that clumping factor in this species, in contrast to S. *aureus*, is an inconstant and perhaps even exceptional feature (8). Subsequently (6, 7), this trait has been recorded as variable. In our sample, we could detect little difference (P > 0.1) between the occurrence of clumping factor in the two species as identified by the Staph-Ident system.

Similarly, the prevalence of beta-toxin (phospholipase C), long appreciated as a characteristic particularly of animal strains, showed no significant difference between the two species (P >> 0.1; Table 5).

The question of differential antimicrobial susceptibility was of particular interest because of its potential reflection on the importance of the precise identification of coagulasepositive species in the clinical laboratory. There was no demonstrably significant difference between our *S. aureus* strains and *S. intermedius* strains with respect to their susceptibilities to antimicrobial drugs (Table 6). β -Lactamase-producing strains in particular appear to occur at the same rate in the two species.

In summary, we found S. intermedius to be the predominant species in canine staphylococcal infections: in those related to the skin, to the apparent exclusion of S. aureus. In cats and horses, either bacterial species could be encountered in clinical infections, whereas in primates and cattle, S. aureus was the predominant form. The most easily observable primary characteristics of animal-pathogenic staphylococci, clumping factor and beta-toxin production, were of no help in distinguishing between the two species. No other differences in tissue and pathogenic specificity between the two bacteria in relation to their various host species could be established (possibly owing in part to the small number within some subsets), and no significant differences in therapeutic drug susceptibilities were observed. The clinical value of routine species identification of coagulase-positive staphylococci, therefore, remains to be established.

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