

Identification of *Staphylococcus* Species of Bovine Origin with the API Staph-Ident System†

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The API Staph-Ident system was evaluated as a means for identifying the species of bovine strains of staphylococci routinely isolated from quarter-milk samples. The species identity of 314 of 581 (54%) isolates of staphylococci was correctly determined by this method. The API Staph-Ident system was more accurate in correctly identifying *Staphylococcus aureus* (93.9%) than in correctly identifying non-*S. aureus* species (41.8%). False identifications of *Staphylococcus epidermidis* and *Staphylococcus hominis* were the main reasons for the incorrect identifications of the non-*S. aureus* species.

Bovine mastitis has been estimated to cost the U.S. dairy industry more than 2 billion dollars a year (11). *Staphylococcus aureus* can be a major cause of mastitis in many dairy herds; however, control programs are available which can be used to reduce the incidence of mastitis caused by *S. aureus*. A recent study (12) indicated that *S. aureus* mastitis was reduced from 16 to 5% by the use of an effective control program; however, more than 23% of the milk samples examined contained pathogens which were classified as *Staphylococcus epidermidis*.

The latest edition of *Bergey's Manual of Determinative Bacteriology* (2) lists *S. aureus*, *S. epidermidis*, and *Staphylococcus saprophyticus* as the only species in the genus *Staphylococcus*. Recently, several new species have been described for this genus and new descriptions have been given for *S. epidermidis* and *S. saprophyticus* (4-6, 10, 14, 15, 22). An increasing number of reports have described the incidence of coagulase-negative staphylococci in infections (1, 3, 7-9, 20, 23). Studies in our laboratory have shown elevated somatic cell counts in milk samples containing coagulase-positive and coagulase-negative staphylococci species that could not be classified according to the descriptions given for the three species listed in *Bergey's Manual* or by the identification scheme of Kloos and Schleifer (13) for human staphylococci isolates.

Miniaturized biochemical test systems have been developed for the rapid identification of several groups of microorganisms. A 24-h system utilizing 20 biochemical tests was developed

for staphylococci by API System, S. A. Montalieu-Vercieu, France (3). In late 1981, Analytab Products, Plainview, N.Y., introduced the API Staph-Ident system which permits the determination of 10 biochemical characteristics after incubation for 5 h. The system is capable of distinguishing all of the human staphylococci species described by Kloos and Schleifer (13) plus two species of veterinary interest, *Staphylococcus hyicus* and *Staphylococcus intermedius*. Between 80 and 96% agreement has been shown between the Staph-Ident system and the biochemical identification scheme of Kloos and Schleifer (13) for clinical staphylococci isolates (1, 7, 8, 17). The purpose of this investigation was to evaluate the API Staph-Ident system for use in the identification to species level of staphylococcal isolates from bovine milk samples.

MATERIALS AND METHODS

Bacterial isolates. During a 20-month period, 581 gram-positive catalase-positive cocci of bovine origin were obtained by our laboratory from several sources. A total of 25 isolates were obtained by swabbing the distal end of the streak canal of 14 cows in the University of Kentucky dairy herd during their dry period. The remaining isolates were obtained from the following sources: 96 were activated from freeze-dried cultures of isolates obtained from quarter foremilk samples from the University of Kentucky dairy herd in 1969 (25, 26), 383 were from quarter foremilk samples obtained from the University of Kentucky dairy herd during the 20-month period of this study, 32 were from two private dairy herds in Kentucky with a high incidence of mastitis (18, herd A; 14, herd B), 37 were from J. W. Pankey, North Louisiana Hill Farm Experiment Station, Homer, La., and 9 were from K. L. Smith, Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster, Ohio. Upon receipt at our laboratory, all milk samples or cultures were streaked on sheep blood esculin agar

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(BEA [21]), and the plates were incubated at 35°C for 24 to 48 h. A single well-isolated colony was picked to brain heart infusion (BHI) broth, incubated at 35°C for 18 h, and stored at 2°C until activated for the API Staph-Ident system and biochemical tests. In addition, each isolate was streaked onto a BHI agar slant and incubated at 35°C for 18 h, and the growth was washed off with sterile 10% skim milk. Skim milk (1 ml) was placed into a sterile Cryotube (GIBCO Laboratories) and stored at -80°C.

API Staph-Ident system. Cultures were transferred up to three times in BHI broth before they were streaked onto BEA. The inocula used to inoculate the strips were prepared from growth on BEA. Recommended procedures of the manufacturer were followed for the preparation of strips and inoculum and for the inoculation, incubation, and reading of the strips. Borderline color reactions that were occasionally obtained with all tests and more often with mannose (MNE) and trehalose (TRE) were considered to be negative in determining the four-digit profile for species identification according to the API Staph-Ident profile register. When a code profile was not found in the profile register, the species identity of the isolate was determined by consulting the API computer center. Since several species were usually given for a profile, the species with the highest probability of occurring for the profile was used to identify the isolate, unless additional tests were recommended for the identification of a species.

Conventional biochemical tests. Inocula for test and test cultures were prepared from the BHI broth used to streak the BEA plate or by activation of the cultures stored at -80°C. The frozen cultures were activated by transferring three times in BHI broth before being used to inoculate test media. The broth cultures were used to determine the following biochemical characteristics: aerobic acid production from arabinose; dextrose; fructose; galactose; lactose; maltose; mannitol; MNE; melezitose; ribose; salicin; sucrose; TRE; turanose; xylitol and xylose (13); anaerobic utilization of mannitol (9); coagulase activity with citrated EDTA rabbit plasma (24); production of heat-sensitive (19) and heat-stable (18, 19) nuclease; lysostaphin susceptibility (19); type of growth in thioglycolate medium (9); alkaline phosphatase activity with p-nitrophenyl phosphate (0.495 mg/ml; Sigma Chemical Co.); growth on Trypticase soy agar containing 7.5, 10, and 15% NaCl (16); nitrate reduction (24), and production of acetyl-methylcarbinol (24). Hemolysis, esculin hydrolysis, and pigmentation were determined by examination of growth on BEA plates. When necessary to separate species, novobiocin susceptibility was determined by using 5- μ g novobiocin sensitivity disks on inoculated Mueller-Hinton agar. Isolates giving zone sizes equal to or less than 11 mm were considered to be novobiocin resistant. Isolates were identified according to a modification of the scheme of Kloos and Schleifer (13) which permitted the identification of *S. hyicus* and *S. intermedius* as well as the human staphylococcal species.

RESULTS

Assuming that the identifications obtained by conventional biochemical tests by the methods

of Kloos and Schleifer (13) were correct, then 314 of 581 bovine staphylococci isolates (54%) were correctly identified by the API Staph-Ident system (Table 1). The accuracy among species varied from 0% for *S. capitis* to 100% for *S. hyicus* and *S. simulans* for isolates identified by the API Staph-Ident system and confirmed by conventional biochemical tests. False identification or failure to identify a species by the API Staph-Ident system generally was due to false negative reactions.

Of 131 *S. aureus* isolates, 123 (93.9%) were correctly identified by the API Staph-Ident system. A positive β -galactosidase reaction resulted in eight *S. aureus* isolates being incorrectly identified as *S. xylosus*. These eight isolates were coagulase positive and negative for xylose utilization, and they were obtained from cows in the same herd.

Approximately 29% of the isolates tested were identified as *S. epidermidis* by the API Staph-Ident system compared with 4.3% by conventional biochemical tests. The API Staph-Ident system identified 7.1% of the isolates as *S. hyicus* compared with 39.4% by the biochemical tests. The API system is unable to differentiate the subspecies within *S. hyicus*. Therefore, the value in Table 1 includes both *S. hyicus* and *S. hyicus* subsp. *chromogenes*.

The biggest problem encountered in this study was the false identification of *S. hyicus* and *S. hyicus* subsp. *chromogenes* as *S. epidermidis*. Only 24 of 171 *S. epidermidis* isolates (14%) were correctly identified by the API Staph-Ident

TABLE 1. Identification of staphylococci of bovine origin with the API Staph-Ident system and with conventional biochemical tests

Staphylococcus species	API		No. identified by biochemical test
	No. identified	No. (%) correctly identified ^a	
<i>S. aureus</i>	124	123 (99.2)	131
<i>S. capitis</i>	11	0 (0)	0
<i>S. epidermidis</i>	171	24 (14.0)	25
<i>S. haemolyticus</i>	40	3 (7.5)	6
<i>S. hominis</i>	45	5 (11.1)	41
<i>S. hyicus</i> ^b	41	41 (100)	229
<i>S. saprophyticus</i>	2	1 (50.0)	1
<i>S. sciuri</i>	20	17 (85.0)	17
<i>S. simulans</i>	54	54 (100)	66
<i>S. warneri</i> ^b	41	24 (58.5)	37
<i>S. xylosus</i>	32	22 (68.8)	23
Unknown			5
Total	581	314 (54.0)	581

^a Species identity was determined by conventional biochemical tests according to Kloos and Schleifer (13).

^b Includes subspecies.

TABLE 2. Frequency of API Staph-Ident four-digit profiles among *Staphylococcus* species of bovine origin

<i>Staphylococcus</i> species	No. of isolates	Correctly identified ^a		Incorrectly identified ^a		Correct species
		API profile	% With profile	API profile	% With profile	
<i>S. aureus</i>	124	7740	64.5	3500	0.8	<i>S. hyicus</i>
		7700	16.1			
		5700	8.9			
		5740	7.3			
		6700	0.8			
		4700	0.8			
		3700	0.8			
<i>S. capitis</i>	11			0040	27.3	<i>S. hominis</i>
					27.3	<i>S. simulans</i>
					18.2	<i>S. haemolyticus</i>
<i>S. epidermidis</i>	171				18.2	<i>S. hyicus</i>
				0240	9.1	<i>S. warneri</i>
		3040	6.4	3040	55.0	<i>S. hyicus</i>
		3000	3.5		0.6	Unknown
		3100	1.8	3440	14.6	<i>S. hyicus</i>
		7040	1.8	7040	4.7	<i>S. hyicus</i>
		2140	0.6	3140	3.5	<i>S. hyicus</i>
				3000	2.9	<i>S. hyicus</i>
				1040	2.3	<i>S. hyicus</i>
				7140	1.2	<i>S. hyicus</i>
<i>S. haemolyticus</i>	40	4440	5.0	7000	0.6	<i>S. hyicus</i>
		0440	2.5	1140	0.6	<i>S. hyicus</i>
				4440	25.0	<i>S. hominis</i>
				4640	19.6	<i>S. warneri</i>
					7.5	<i>S. hominis</i>
				0440	7.5	<i>S. hominis</i>
				0061	7.5	<i>S. simulans</i>
				4400	5.0	<i>S. hominis</i>
				0640	2.5	<i>S. hominis</i>
					2.5	<i>S. warneri</i>
				4660	2.5	<i>S. warneri</i>
					2.5	Unknown
				1440	2.5	<i>S. hyicus</i>
				0041	2.5	<i>S. simulans</i>
				0060	2.5	<i>S. simulans</i>
<i>S. hominis</i>	45	2440	4.4	0460	2.5	<i>S. simulans</i>
		2000	2.2	2040	62.2	<i>S. hyicus</i>
		2040	2.2		4.4	<i>S. simulans</i>
		2400	2.2	2440	15.6	<i>S. hyicus</i>
				2000	2.2	<i>S. epidermidis</i>
<i>S. hyicus</i>	41			0400	2.2	<i>S. haemolyticus</i>
		3540	68.3	2400	2.2	<i>S. hyicus</i>
		1540	14.6			
		7540	12.2			
		2540	4.9			
<i>S. saprophyticus</i>	2	2401	50.0	2601	50.0	<i>S. xylosum</i>
<i>S. sciuri</i>	20	5710	60.0	4650	10.0	<i>S. warneri</i>
		4710	15.0	4510	5.0	Unknown
		4610	10.0			
<i>S. simulans</i>	54	2461	31.5			
		2061	29.6			
		2041	13.0			
		6061	9.3			
		6461	3.7			
		2421	1.9			
		6041	1.9			
		6441	1.9			
<i>S. warneri</i>	25	6640	12.0	6440	28.0	<i>S. hominis</i>
		6440	12.0	6640	16.0	<i>S. hominis</i>
		6650	4.0	7440	8.0	<i>S. hyicus</i>

TABLE 2—Continued

Staphylococcus species	No. of isolates	Correctly identified ^a		Incorrectly identified ^a		Correct species
		API profile	% With profile	API profile	% With profile	
<i>S. warneri</i> subsp. 1	16	6040	4.0	4500	4.0	<i>S. hominis</i>
		4240	4.0	6400	4.0	<i>S. hominis</i>
		6660	43.8	4200	4.0	Unknown
		6060	25.0	6660	6.3	<i>S. hominis</i>
		2620	12.5			
		2420	6.3			
<i>S. xylosus</i>	32	2020	6.3			
		6021	9.4	7701	21.9	<i>S. aureus</i>
		6421	6.3	7601	3.1	<i>S. aureus</i>
		6721	6.3	6421	3.1	<i>S. simulans</i>
		7721	6.3	6711	3.1	Unknown
		7731	6.3			
		4721	3.1			
		6301	3.1			
		6321	3.1			
		6461	3.1			
		7001	3.1			
		7021	3.1			
		7421	3.1			
		7431	3.1			
		7521	3.1			
		7621	3.1			
6731	3.1					

^a Species identity was determined by conventional biochemical tests according to the scheme of Kloos and Schleifer (13).

system. Of the 147 isolates incorrectly identified as *S. epidermidis*, 146 were identified as *S. hyicus* or its subspecies by biochemical tests. False identifications were due mainly to negative MNE and TRE utilizations by *S. hyicus* and its subspecies that resulted in code profiles for *S. epidermidis*. Most of the false identifications obtained were for pigmented isolates that were identified as *S. hyicus* subsp. *chromogenes* by biochemical tests.

A total of 36 *S. hyicus* were incorrectly identified as *S. hominis* owing to low percentages of positive reactions for alkaline phosphatase, MNE utilization, and TRE utilization. A negative urease reaction or a positive β -glucosidase reaction resulted in 37 isolates being incorrectly identified as *S. haemolyticus*, rather than as *S. hominis*, *S. warneri*, and *S. simulans*.

The API four-digit profiles obtained for each species are shown in Table 2. A total of 89 different profiles were obtained in this study, of which 79.8% were unique for a single species and 16.9% overlapped two species. Approximately 2% of the profiles overlapped three species and 1.1% of the profiles overlapped four species.

Approximately 78% of the isolates (451) yielded profiles that were included in the profile register of the manufacturer. The remaining 130

isolates were identified by the API computer center. Unless additional tests were recommended for the identification of a species, the species with the highest probability of occurring for a profile was used to identify the isolate. Percentages of species requiring the use of the API computer center for their identification were: *S. warneri* subsp. 1 (100%), *S. warneri* (96%), *S. haemolyticus* (45%), *S. sciuri* (25%), *S. hominis* (22.2%), *S. epidermidis* (18.7%), *S. hyicus* (17.1%), *S. simulans* (16.7%), and *S. aureus* (1.6%).

The number of API four-digit profiles obtained from each species ranged from 2 for *S. capitis* and *S. saprophyticus* to 19 for *S. xylosus*. The number of profiles that gave correct species identification ranged from 0 for *S. capitis* to 16 for *S. xylosus*.

The *S. aureus* isolates that were correctly identified had seven different code profiles, with 7740 (64.5%), 7700 (16.1%), 5700 (8.9%), and 5740 (7.3%) accounting for all but three isolates. One *S. aureus* isolate with profile 3500 was identified by biochemical tests as a coagulase-negative *S. hyicus*. The 11 isolates with API code profiles for *S. capitis* (0040 and 0240) were identified as *S. hominis*, *S. simulans*, *S. haemolyticus*, *S. hyicus*, and *S. warneri*. Of the 147 *S. epidermidis* that were incorrectly identified,

63.2% had the same API code profiles (3000, 3040, and 7040) as isolates correctly identified as *S. epidermidis*. Isolates with API code profiles (0440 and 4440) were identified as *S. haemolyticus* and *S. hominis*. Isolates incorrectly identified as *S. haemolyticus* were identified as *S. hominis* (4440, 4640, 0440, 0640, and 4400), *S. warneri* (4640, 0640, and 4660), *S. simulans* (0061, 0041, 0060, and 0460), and *S. hyicus* (1440). All API code profiles correctly identified as *S. hominis* also had isolates that were identified as *S. hyicus* (2040, 2440, and 2400), *S. simulans* (2040), and *S. epidermidis* (2000).

The identification of *S. warneri* (96%) and *S. warneri* subsp. 1 (100%) was based on assistance from the API computer center. API code profiles 6440 and 6640 were identified biochemically as *S. warneri* and *S. hominis*, whereas 6660 was identified as *S. warneri* subsp. 1 and *S. hominis*. API code profiles 7440, 6400, and 4500 were found to be *S. hyicus* and *S. hominis* rather than *S. warneri*. *S. simulans* was found to have the same API code profile as *S. xylosus* (6421). *S. xylosus* with API code profiles 7601 and 7701 were found to be coagulase positive and negative for xylose utilization. These isolates were identified as *S. aureus*.

A summary of the percentages of isolates in each species, as determined by conventional biochemical tests, giving positive results for each of the biochemical tests in the API Staph-Ident strip and the additional test for coagulase activity is given in Table 3. The API strip is unable to differentiate the subspecies within *S. hyicus*. For comparison purposes, we divided the isolates identified as *S. hyicus* into those that were nonpigmented (*S. hyicus*) and pigmented (*S. hyicus* subsp. *chromogenes*). Based on our results, the percentages of positive reaction for the biochemical tests in the API strip differ between *S. hyicus* and *S. hyicus* subsp. *chromogenes*, and values tended to be similar for *S. epidermidis* and *S. hyicus* subsp. *chromogenes*. *S. warneri* and *S. warneri* subsp. 1 are combined in the table. The main difference between the two is that 100% of the isolates identified as *S. warneri* were negative for β -glucuronidase, whereas 100% of the isolates identified as *S. warneri* subsp. 1 were positive. Percentages of positive reactions obtained for the biochemical tests in the API strips tended to be similar to the values reported by Kloos and Wolfshohl (17) and the manufacturer of the API Staph-Ident system for *S. aureus*, *S. epidermidis*, *S. sciuri*, *S. warneri*, and *S. xylosus*. Some minor differences obtained were negative results for alkaline phosphatase (*S. simulans* and *S. xylosus*), MNE utilization (*S. haemolyticus* and *S. simulans*), and β -glucuronidase (*S. haemolyticus* and *S. hyicus*) and higher positive results for urea utili-

zation (*S. hyicus*), β -glucosidase (*S. hyicus* and *S. simulans*), MNE utilization (*S. hominis*), and arginine utilization (*S. hominis* and *S. warneri*). Lower positive results were obtained for urea utilization (*S. hominis*), β -glucosidase (*S. hyicus*), MNE utilization (*S. hyicus*), and TRE utilization (*S. haemolyticus*, *S. hyicus*, and *S. simulans*). Some of the differences obtained may be due to the small number of isolates in some species.

DISCUSSION

A simple, rapid, and accurate method for determining the species identities of bovine isolates of staphylococci would have value in identifying species associated with varying degrees of clinical severity of bovine mastitis and with elevated somatic cell counts. Such a method would permit the veterinarian to rapidly determine the *Staphylococcus* species responsible for mastitis rather than having to submit samples to an animal diagnostic laboratory for analysis.

The purpose of this study was to determine the accuracy of a rapid, miniaturized biochemical system (API Staph-Ident) that was designed for use with human clinical isolates as a means for identifying the species of bovine isolates of staphylococci.

The accuracy that we obtained in correctly identifying bovine isolates as *S. aureus* (93.9%) by the API Staph-Ident system was similar to the values reported in studies with human isolates (1, 7, 8, 17). However, owing to our low accuracy in correctly identifying non-*S. aureus* species (41.8%), the overall accuracy of 54% that we obtained for the 581 bovine isolates tested was much lower than the 80 to 96% reported by other researchers for human isolates (1, 7, 8, 17). The proportion of particular species comprising the isolates being tested will affect the percentage agreement between the API Staph-Ident system and conventional biochemical tests. Our highest agreements were obtained for sources that gave a majority of isolates that were identified as *S. aureus*. The lowest agreements were obtained for sources that gave a variety of non-*S. aureus* species (especially *S. hyicus* subsp. *chromogenes*).

False identifications of the non-*S. aureus* species by the API system were due to incomplete color changes, especially with MNE and TRE. After 5 h of incubation, the color of some sugars was red-orange or orange, and slight color changes were observed for alkaline phosphatase, β -glucosidase, and β -glucuronidase. According to the directions of the manufacturer for the interpretation of results, these color changes are to be considered negative. Unlike the results of Doern et al. (7), incubation for an additional 19 h did not result in positive color changes. In

fact, many times the color of a sugar changed back to the original red color. Our overall accuracy would have approached the values reported in other studies if we had considered some of the incomplete color changes positive rather than negative. This would have been especially true for the incomplete color changes obtained for MNE and TRE by *S. hyicus* and its subspecies. Our study indicated that unless positive reactions are definite for the biochemical tests of the API Staph-Ident system, problems will be encountered in the proper identification of some species (*S. epidermidis*, *S. hyicus*, *S. hyicus* subsp. *chromogenes*, *S. haemolyticus*, and *S. hominis*) of bovine origin. Based on our experience with bovine isolates, some type of color chart would be helpful in standardizing the test, especially in deciding when to record an incomplete color change positive. The color chart may be more useful with nonhuman isolates, since reactions of nonhuman isolates appear more variable. The color change for a reaction to be considered positive for the API Staph-Ident system may depend on the source of the isolate.

The reason for the large number of false identifications of non-*S. aureus* species by the API Staph-Ident system is unknown. One problem may be the use of a system designed for the rapid identification of human isolates to identify bovine isolates. Differences in biochemical characteristics between human and bovine isolates may result in different rates of substrate utilization after 5 h of incubation. As a result of these differences, code profiles are obtained that correctly identify human isolates but incorrectly identify bovine isolates.

Methods currently being used to control mastitis may affect some of the biochemical characteristics used for the identification of staphylococci. Weckback and Langlois (26) found significant differences between some biochemical characteristics of isolates from teats routinely dipped after each milking with an iodophor teat dip and those not dipped. Biochemical characteristics normally associated with staphylococci and used in their identification were affected by the teat dip. They did not indicate whether the differences were due to the effect of the teat dip on the organisms or to the selection of populations in the dipped teats that differed biochemically from the nondipped teats.

Like our study, other studies (7, 8, 17, 23) also used the biochemical identification scheme of Kloos and Schleifer (13) for human isolates to evaluate the accuracy of the API Staph-Ident system. Except for Kloos and Wolfshohl (17), other researchers (7, 8, 23) used human isolates to evaluate the API Staph-Ident system. Therefore, the other studies did not encounter false identification of *S. hyicus* and *S. hyicus* subsp.

TABLE 3. Differentiation of *Staphylococcus* species by API Staph-Ident system biochemical tests^a

Staphylococcus species	No. of isolates	% of positive reaction in API Staph-Ident system test ^b											% of positive reaction in coagulase test
		PHS	URE	GLS	MNE	MAN	TRE	SAL	GLC	ARG	NGP		
<i>S. aureus</i>	131	98.5	84.0	99.2	99.2	100	100	0	0	67.9	6.1	100	
<i>S. epidermidis</i>	25	92.0	100	12.0	16.0	0	0	0	0	60.0	0	0	
<i>S. haemolyticus</i>	6	0	0	33.3	0	0	66.7	0	0	83.3	0	0	
<i>S. hominis</i>	41	0	43.9	70.0	2.4	22.0	87.8	0	2.4	83.4	0	0	
<i>S. hyicus</i> ^c	229	82.9	94.3	7.9	22.4	0	34.2	0	0	96.9	0	2.2	
<i>S. hyicus</i> subsp. <i>chromogenes</i>	23	100	69.6	4.3	73.9	0	69.6	0	0	95.7	0	21.7	
<i>S. saprophyticus</i>	206	80.6	96.6	8.3	16.5	0	30.1	0	0	96.6	0	0	
<i>S. sciuri</i>	1	100	100	0	0	0	100	0	0	0	100	0	
<i>S. simulans</i>	17	70.6	0	100	88.2	100	100	0	0	0	0	0	
<i>S. warneri</i> ^c	66	0	86.4	15.2	0	0	40.9	0	71.2	97.0	89.4	0	
<i>S. xylosox</i>	37	0	62.2	83.8	0	73.0	78.4	8.1	43.2	89.2	0	0	
	23	0	95.7	95.7	47.8	52.2	69.6	17.4	87.0	4.3	100	0	

^a Species identification based on conventional biochemical tests. Of 581 isolates, 5 were not identified.
^b PHS, Alkaline phosphatase; URE, urease; GLS, β-glucosidase; MNE, mannitol; SAL, salicin; GLC, β-glucuronidase; ARG, arginine; NGP, β-galactosidase.
^c Includes subspecies.

chromogenes as *S. epidermidis*. This is not surprising since both *S. hyicus* and *S. hyicus* subsp. *chromogenes* are considered to be primarily associated with animals (6). Both species have been isolated from cows with mastitis (6). The percentages of positive reactions for the biochemical tests in the API Staph-Ident strip tended to be similar for *S. epidermidis* and *S. hyicus* subsp. *chromogenes* (Table 3). The biggest differences were for TRE and arginine utilization. Since *S. hyicus* subsp. *chromogenes* is common to bovine milk and gives reactions similar to *S. epidermidis* on the API strip, it is not surprising that false identifications were obtained. To prevent false identifications of bovine isolates, additional tests (DNase, Voges-Proskauer, maltose utilization, ribose utilization) are necessary to correctly identify *S. hyicus* and its subspecies. Our percent agreement between the API Staph-Ident system and conventional biochemical tests was markedly improved when we used one or more of these tests with the API Staph-Ident system.

The code profiles obtained were similar to those reported by others (7, 17). The majority of *S. aureus* isolates yielded a 7740 profile (64.5%). The most common profile for *S. epidermidis* was 3040 (64%); however, based on biochemical tests, 89.5% of the isolates with a 3040 profile were identified as *S. hyicus*. Likewise, the 7040 profile observed most frequently for *S. epidermidis* by Doern et al. (7) generally was found in our study to be *S. hyicus*. Other code profiles obtained were similar to those reported by Kloos and Wolfshohl (17).

The results of this study indicated that *S. aureus* of bovine origin could be accurately identified by the API Staph-Ident system. However, only 41.8% of the non-*S. aureus* species were correctly identified. Since several non-*S. aureus* species have been associated with mastitis and elevated somatic cell counts, the API Staph-Ident system as it is currently manufactured or used for clinical human isolates appears to be of limited value in determining the identity of non-*S. aureus* species of bovine origin.

LITERATURE CITED

- Aldridge, K. E., C. W. Stratton, L. S. Patterson, M. E. Evans, and R. L. Hodges. 1983. Comparison of the Staph-Ident system with a conventional method for species identification of urine and blood isolates of coagulase-negative staphylococci. *J. Clin. Microbiol.* **17**:516-520.
- Baird-Parker, A. C. 1974. Gram-positive cocci. *Famili I. Micrococcaceae*, p. 478-490. In R. E. Buchanan and N. E. Gibbon (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Brun, Y., J. Fleurette, and F. Orey. 1978. Micromethod for biochemical identification of coagulase-negative staphylococci. *J. Clin. Microbiol.* **8**:503-508.
- Devriese, L. A. 1977. Isolation and identification of *Staphylococcus hyicus*. *Am. J. Vet. Res.* **38**:787-792.
- Devriese, L. A., and J. Derycke. 1979. *Staphylococcus hyicus* in cattle. *Res. Vet. Sci.* **26**:356-358.
- Devriese, L. A., V. Hajek, P. Oeding, S. A. Meyer, and K. H. Schleifer. 1978. *Staphylococcus hyicus* (Sompolsky, 1953) comb. nov. and *Staphylococcus hyicus* subsp. *chromogenes* subsp. nov. *Int. J. Syst. Bacteriol.* **28**:482-490.
- Doern, G. V., J. E. Earls, P. A. Jeznach, and D. S. Parker. 1983. Species identification and biotyping of staphylococci by the API Staph-Ident system. *J. Clin. Microbiol.* **17**:260-263.
- Gemmel, C. G., and J. E. Dawson. 1982. Identification of coagulase-negative staphylococci with API Staph system. *J. Clin. Microbiol.* **16**:874-877.
- Gunn, B. A., F. L. Singleton, E. R. Peepler, R. R. Colwell, J. K. Keiser, and C. O. Kapfer. 1981. Comparison of methods for identifying *Staphylococcus* and *Micrococcus* spp. *J. Clin. Microbiol.* **14**:195-200.
- Hajek, V. 1976. *Staphylococcus intermedius*, a new species isolated from animals. *Int. J. Syst. Bacteriol.* **26**:401-408.
- Jasper, D. E., J. S. McDonald, R. D. Mochrie, W. N. Philpot, R. J. Farnsworth, and S. B. Spencer. 1982. Bovine mastitis research: needs, funding and source of support. In Proceedings of the 21st Annual Meeting of the National Mastitis Council, p. 184-193. National Mastitis Council, Inc., Washington, D.C.
- Jones, G. M., R. E. Pearson, C. W. Heald, and W. E. Vinson. 1982. Milk loss, somatic cell counts and udder infections in Virginia herds. In Proceedings of the 21st Annual Meeting of the National Mastitis Council, p. 31-36. National Mastitis Council, Inc., Washington, D.C.
- Kloos, W. E., and K. H. Schleifer. 1975. Simplified scheme for routine identification of human *Staphylococcus* species. *J. Clin. Microbiol.* **1**:82-88.
- Kloos, W. E., and K. H. Schleifer. 1975. Isolation and characterization of staphylococci from human skin. II. Descriptions of four new species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus simulans*. *Int. J. Syst. Bacteriol.* **25**:62-79.
- Kloos, W. E., K. H. Schleifer, and R. F. Smith. 1976. Characterization of *Staphylococcus sciuri* sp. nov. and its subspecies. *Int. J. Syst. Bacteriol.* **26**:22-37.
- Kloos, W. E., T. G. Tornabene, and K. H. Schleifer. 1974. Isolation and characterization of micrococci from human skin, including two new species: *Micrococcus lylae* and *Micrococcus kristinae*. *Int. J. Syst. Bacteriol.* **24**:79-101.
- Kloos, W. E., and J. F. Wolfshohl. 1982. Identification of *Staphylococcus* species with the API Staph-Ident system. *J. Clin. Microbiol.* **16**:509-516.
- Lachica, R. V. F., C. Genigeorgis, and P. D. Hoepflich. 1971. Metachromatic agar-diffusion method for detecting staphylococcal nuclease activity. *Appl. Microbiol.* **21**:585-587.
- Lachica, R. V. F., P. D. Hoepflich, and C. Genigeorgis. 1971. Nuclease production and lysostaphin susceptibility of *Staphylococcus aureus* and other catalase-positive cocci. *Appl. Microbiol.* **21**:823-826.
- Marsik, F. J., and S. Brake. 1982. Species identification and susceptibility to 17 antibiotics of coagulase-negative staphylococci isolated from clinical specimens. *J. Clin. Microbiol.* **15**:640-645.
- National Mastitis Council. 1981. Microbiological procedures for use in the diagnosis of bovine mastitis. National Mastitis Council, Inc., Washington, D.C.
- Schleifer, K. H., and W. E. Kloos. 1975. Isolation and characterization of staphylococci from human skin. I. Amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus* and *Staphylococcus xylosum*. *Int. J. Syst. Bacteriol.* **25**:50-61.
- Sewell, C. M., J. E. Clairidge, E. J. Young, and R. K. Guthrie. 1982. Clinical significance of coagulase-negative

- staphylococci. *J. Clin. Microbiol.* **16**:236-239.
24. **Smitbert, R. M., and N. E. Krieg.** 1981. General characterization, p. 409-443. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. Krieg, and G. B. Phillips (ed.), *Manual of methods for general bacteriology*. American Society for Microbiology, Washington, D.C.
25. **Weckbach, L. S., and B. E. Langlois.** 1976. Classification by numerical taxonomy of staphylococci isolated from the bovine udder. *J. Milk Food Technol.* **39**:246-249.
26. **Weckbach, L. S., and B. E. Langlois.** 1972. The effects of the teat dip "Bovadine" on some characteristics of staphylococci isolated from the bovine udder. *J. Milk Food Technol.* **35**:501-502.