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Identification of *Staphylococcus* Species of Bovine Origin with the DMS Staph-Trac System†

BRUCE E. LANGLOIS,* ROBERT J. HARMON, AND KATHERINE AKERS

Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546-0215

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The DMS Staph-Trac 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 83 of 91 (91.2%) isolates of staphylococci was correctly determined by this method. One isolate could not be identified by this system. The Staph-Trac system was able to distinguish between *Staphylococcus hyicus* and *Staphylococcus epidermidis*. We obtained a higher percentage of correct identifications with the DMS Staph-Trac system (91.2%) than we did in a previous study with the API Staph-Ident system (45.1%), using the same isolates (Langlois et al., J. Clin. Microbiol. 18:1212–1219, 1983).

Miniaturized biochemical test systems have been developed for the rapid identification of several groups of microorganisms. A 24-h system (Staph Strip) utilizing 20 biochemical tests, including a negative control, was developed for staphylococci by API Systems S. A., Montalieu-Vercieu, France (1). In 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 (4). The API Staph-Ident system is capable of distinguishing all of the human staphylococci species described by Kloos and Schleifer (3) plus two species of veterinary interest, *Staphylococcus hyicus* and *Staphylococcus intermedius*. Recently, DMS Laboratories, Flemington, N.J., began distribution in the United States of the 24-h, 20-biochemical-test system developed by API Systems under the name DMS Staph-Trac. The DMS Staph-Trac system is recommended for the identification of *Staphylococcus aureus* and coagulase-negative staphylococci species. This system will not distinguish *S. aureus* from *S. intermedius*. Maddux and Koehne (6) reported that the 24-h Staph Strip system could be used for the identification of *S. hyicus* isolated from swine. Giger et al. (2) reported that the DMS Staph-Trac system correctly identified 88.3% of human clinical isolates of coagulase-negative staphylococci compared with a 79.2% correct identification of the same isolates by the API Staph-Ident system. The purpose of this study was to evaluate the DMS Staph-Trac system for use in the identification to species level of staphylococcal isolates from bovine milk samples and to compare these results with those we obtained in a previous study with the API Staph-Ident system (5).

MATERIALS AND METHODS

Bacterial isolates. Of 581 isolates used in a previous study (5), 96 were selected for use in this study. These isolates represented each species identified by the API Staph-Ident system in that study, with an emphasis on selecting isolates that the API Staph-Ident system had difficulty in identifying correctly. Also an *S. intermedius* isolate from a dry-cured ham was included in this study. The 97 isolates had been stored in 10% skim milk at −80°C until activated for use in this study.

DMS Staph-Trac system. Cultures were transferred three times in brain heart infusion broth before they were streaked onto P agar (7). Recommended procedures of the manufacturer were followed for the preparation of strips and inoculum and for the inoculation, incubation, and reading of the strips. A bacterial suspension equal to a no. 2 McFarland Standard, as specified for the lot of strips used, was prepared in DMS Staph-Trac broth, using growth from a P agar plate incubated at 37°C for 24 h. The suspension was used to inoculate the 20 cupules (19 biochemical tests and a control) contained on the DMS Staph-Trac strips. The strips were incubated at 37°C for 24 h and read according to the directions of the manufacturer. Reagents required to determine reduction of nitrate to nitrite, production of acetylmethylcarbinol, and alkaline phosphatase were purchased from DMS Laboratories. Reading of the strips resulted in a seven-digit numerical code which was used for species identification according to code profiles listed in the DMS Staph-Trac identification code book. When a code profile

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

Staphylococcus species	DMS Staph-Trac[a]		No. identified by biochemical tests[b]
	No. identified	No. (%) correctly identified[b]	
S. aureus	11	10 (90.9)	10
S. epidermidis	11	11 (100)	11
S. hominis	22	18 (81.8)	19
S. hyicus	21	21 (100)	26
S. saprophyticus	1	1 (100)	1
S. sciuri	3	3 (100)	3
S. simulans	7	6 (85.7)	6
S. warneri	7	6 (85.7)	6
S. xylosus	7	7 (100)	8
S. intermedius	0	0	1
Unknown	1[c]		6[d]
Total	91	83 (91.2)	97

[a] Six isolates could not be identified by the scheme of Kloos and Schleifer (3), and these six isolates are not included in the DMS Staph-Trac results.
[b] Species identity was determined by conventional biochemical tests by Kloos and Schleifer (3).
[c] Seven-digit profile number not in code book or in computer data bank or unable to identify by use of recommended supplementary tests.
[d] Unable to identify by scheme of Kloos and Schleifer (3).

* Corresponding author.
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was not found in the code book, the species identity of the isolate was determined by consulting the DMS computer service. When a profile resulted in a low discrimination between two or more species, supplementary tests recommended in the code book were used to determine the identity of the isolate.

Conventional biochemical tests. Procedures described previously (5) were used to determine the following biochemical

TABLE 2. Frequency of DMS Staph-Trac seven-digit profiles among *Staphylococcus* species of bovine origin

Staphylococcus species	No. of isolates	Correctly identified[a]		Incorrectly identified[a]		Correct species
		DMS profile	No. with profile	DMS profile	No. with profile	
S. aureus	11	6 736 150	3	6 716 153	1	*S. intermedius*
		6 736 152	3			
		6 736 153	2			
		6 736 113	1			
		6 736 151	1			
S. epidermidis	11	6 706 113	6			
		6 606 112	1			
		6 702 113	1			
		6 704 110	1			
		6 704 112	1			
		6 606 113	1			
S. hominis	23	6 612 111	4	6 716 013	2	*S. hyicus*
		6 232 111	3	6 716 113	2	*S. hyicus*
		6 232 113	2	6 236 111	1	Unknown
		6 612 113	2			
		6 612 101	2			
		6 212 111	1			
		6 416 013	1			
		6 632 113	1			
		6 712 152	1			
		6 612 103	1			
S. hyicus	21	6 716 053	6			
		6 516 013	3			
		6 516 053	3			
		6 416 013	2			
		6 516 113	2			
		6 116 053	1			
		6 506 051	1			
		6 516 151	1			
		6 516 153	1			
		6 536 111	1			
S. saprophyticus	1	6 670 112	1			
S. sciuri	3	6 736 050	2			
		6 336 450	1			
S. simulans	7	6 512 151	2	6 532 112	1	*S. xylosus*
		6 412 053	1			
		6 412 153	1			
		6 432 053	1			
		6 530 053	1			
S. warneri	9	6 232 111	5	6 230 113	1	*S. hominis*
		6 230 112	1	6 310 114	1	Unknown
				6 630 111	1	Unknown
S. xylosus	8	6 736 512	2	6 132 110	1	Unknown
		6 726 552	1			
		6 732 552	1			
		6 734 552	1			
		6 736 552	1			
		6 776 552	1			
S. capitis	1			6 102 103	1	Unknown
Unknown[b]	2			6 716 113	1	*S. hyicus*
				6 632 105	1	Unknown

[a] Species identity was determined by conventional biochemical tests by the scheme of Kloos and Schleifer (3).
[b] Seven-digit profile number not in DMS Staph-Trac code book or in computer data bank or unable to identify by use of recommended supplementary tests.

characteristics: aerobic acid production from arabinose, dextrose, fructose, galactose, lactose, maltose, mannitol, mannose, melezitose, ribose, salicin, sucrose, trehalose, turanose, xylitol, and xylose; anaerobic utilization of mannitol; tube coagulase; production of heat-sensitive and heat-stable nuclease; lysostaphin susceptibility; type of growth in thioglycolate medium; alkaline phosphatase activity; growth on 7.5, 10, and 15% NaCl; nitrate reduction; production of acetylmethylcarbinol; hemolysis; esculin hydrolysis; pigmentation; and novobiocin susceptibility. Isolates were identified by a modification of the scheme of Kloos and Schleifer (3) which permitted the identification of *S. hyicus* and *S. intermedius* as well as the human staphylococcal species.

RESULTS AND DISCUSSION

Six of the isolates tested could not be identified by biochemical tests by the scheme of Kloos and Schleifer (3). These isolates do not appear to be typical of any recognized *Staphylococcus* species. Since they may represent new, undescribed organisms, work is presently under way in our laboratory to determine whether these isolates represent new *Staphylococcus* species or additional subspecies of existing *Staphylococcus* species.

The six isolates that were not identified were not included in the results presented for the DMS Staph-Trac system (Table 1). The six isolates not identified by biochemical tests had DMS code profiles (Table 2) that identified them as *Staphylococcus warneri* (2), *Staphylococcus hominis* (1), *Staphylococcus xylosus* (1), *Staphylococcus capitis* (1), and unknown (1). Assuming that the identifications obtained by conventional biochemical tests by the methods of Kloos and Schleifer (3) were correct, then 83 of 91 bovine isolates (91.2%) were correctly identified by the DMS Staph-Trac system. The accuracy among species varied from 81.8% for *S. hominis* to 100% for *Staphylococcus epidermidis*, *S. hyicus*, *Staphylococcus saprophyticus*, *Staphylococcus sciuri*, and *S. xylosus*. In a previous study (5) using 581 isolates of bovine origin, we obtained a 45.1% correct identification for these 91 isolates with the API Staph-Ident system.

S. aureus cannot be distinguished from *S. intermedius* with the DMS Staph-Trac system unless protein A is determined. Since we did not determine protein A, the one isolate identified as *S. intermedius* by biochemical tests is included with *S. aureus*.

Four *S. hyicus* isolates were incorrectly identified as *S. hominis* due to positive maltose reactions on the strips, and one *S. hyicus* isolate was not identified by the DMS Staph-Trac system. Biochemical tests indicated that these isolates were maltose-positive *S. hyicus*. Unpublished data from our laboratory indicate that 12.5 and 27.2% of bovine isolates identified as *S. hyicus* subsp. *hyicus* and *S. hyicus* subsp. *chromogenes*, respectively, were maltose-positive isolates. According to the percentage of isolates giving positive reactions listed in the DMS code book, 1% or less of *S. hyicus* isolates are maltose positive. Three *S. hyicus* isolates had the same seven-digit profile (6 716 113). Two isolates had characteristics that fit the results of the supplementary tests for *S. hominis*, whereas the third isolate could not be identified, using the supplementary tests.

One *S. hominis* isolate was incorrectly identified as *S. warneri* due to negative lactose utilization and failure to reduce nitrate. One *S. xylosus* isolate was incorrectly identified as *S. simulans* because of a false-negative xylose utilization reaction on the strip. This isolate was considered to be *S. xylosus* by the biochemical tests.

The DMS Staph-Trac seven-digit profiles obtained for the 97 isolates are shown in Table 2. A total of 58 different profiles were obtained in this study, of which 67.2% were unique for a single isolate, and 7% of the profiles were obtained by four or more isolates. Only one profile overlapped a single species. Three isolates identified as *S. hyicus* by biochemical tests had profile 6 716 113; based upon the results of the supplementary tests, two of the isolates were identified as *S. hominis* and one could not be identified by these tests.

The number of seven-digit profiles obtained for each species ranged from 1 for *S. capitis* and *S. saprophyticus* to 13 for *S. hominis*. The number of profiles that gave correct species identification ranged from 0 for *S. capitis* to 10 for *S. hominis* and *S. hyicus*. The *S. aureus* isolates that were correctly identified had five different code profiles, with 6 736 150 (30%), 6 736 152 (30%), and 6 736 113 (20%) accounting for all but two isolates. One isolate with code profile 6 716 153 was identified by biochemical tests as *S. intermedius*. This was not unexpected since the profiles obtained with the Staph-Trac system cannot distinguish between *S. aureus* and *S. intermedius*. Determination of protein A is the procedure recommended to distinguish these two species, and it was not determined. The 4 *S. hyicus* isolates incorrectly identified as *S. hominis* had code profiles 6 716 013 and 6 716 113. One *S. hominis* isolate (6 230 113) was incorrectly identified as *S. warneri*, and one *S. xylosus* isolate (6 532 112) was incorrectly identified as *Staphylococcus simulans*.

The results of this study indicate that bovine isolates of staphylococci can be accurately identified to species level with the DMS Staph-Trac system. Our results were similar to those obtained by Giger et al. (2). The DMS Staph-Trac system gave a higher percentage of correct identification (91.2%) of bovine isolates of staphylococci than we obtained in a previous study (5) with the API Staph-Ident system (45.1%), using the same isolates. The greater accuracy was due to the ability of the DMS Staph-Trac system to distinguish between *S. epidermidis* and *S. hyicus* isolates. The API Staph-Ident system gave false identification of *S. hyicus* as *S. epidermidis*, with only 14% of the isolates being correctly identified as *S. epidermidis*. Apparently, the greater number of tests in the DMS Staph-Trac system (19 versus 10) and the longer incubation time (24 versus 5 h) make this system more suitable for the accurate identification to species level of bovine isolates of staphylococci. The results obtained with the DMS Staph-Trac system showed excellent agreement with the conventional biochemical tests results. Also, the inclusion of a negative control results in a more accurate interpretation of the reactions, which tends to reduce the number of false-negative and false-positive reactions that are recorded.

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