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Evaluation of the Staph-Ident and STAPHase Systems for Identification of Staphylococci from Bovine Intramammary Infections

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The Staph-Ident and STAPHase systems (Analytab Products, Plainview, N.Y.) were compared with conventional methods for identification of staphylococci isolated from bovine intramammary infections. Adjunct testing by colony morphology, pigmentation, and biochemical tests was conducted to resolve discrepant identifications. The initial accuracies of the conventional scheme and Staph-Ident were 92.1 and 89.2%, respectively. *Staphylococcus hyicus* subsp. *chromogenes* could not be identified by means of the Staph-Ident test, but the addition of pigment production as a key character permitted identification of most strains. The final accuracy of the Staph-Ident was 94.3%. The STAPHase system was as accurate as the conventional tube coagulase method. The Staph-Ident and STAPHase systems are acceptable alternatives to conventional methods for identification of staphylococcal species isolated from bovine intramammary infections.

Bovine mastitis is a disease of great economic importance to the dairy industry, with national losses approaching two billion dollars per year (26). Members of the family *Micrococcaceae* are the organisms most frequently isolated from bovine udder skin, teats, and milk (4, 7, 11, 16). Of this group, *Staphylococcus aureus* is considered a primary pathogen of the bovine mammary gland, whereas *Staphylococcus epidermidis* and *Micrococcus* spp. are believed to be nonpathogenic (4, 7, 28). Recent investigations (2, 18, 19) have revealed that staphylococci other than *S. aureus* may be important intramammary pathogens and emphasize the need for accurate, rapid identification methods for mastitis management and epizootiological studies.

Current procedures (5, 6, 17, 20) for classification of gram-positive, catalase-positive cocci identify β-hemolytic, coagulase-positive cocci as *S. aureus*. Weakly hemolytic or nonhemolytic, coagulase-negative cocci are identified as *S. epidermidis* and *Micrococcus* spp.

Recent taxonomic studies (22, 24, 25) provided new insight into the physiochemical characteristics of *Micrococcaceae*. Subsequently, descriptions of several new coagulase-positive and coagulase-negative staphylococcal species were published as well as more definitive descriptions of previously established species (8, 10, 12, 14, 23).

Kloos and Schliefer (13) developed a simplified scheme for the identification of staphylococci. Species of veterinary importance described after this scheme was devised included *Staphylococcus intermedius*, *Staphylococcus hyicus* subsp. *hyicus*, *Staphylococcus hyicus* subsp. *chromogenes*, and *Staphylococcus sciuri*. Devriese (7) developed a scheme for identification of gram-positive, catalase-positive, clumping factor-negative cocci from bovine intramammary infections (IMI), but did not include the coagulase-positive species *S. intermedius*. Both schemes require numerous media, are labor intensive, and have extended incubation periods that limit usefulness in the diagnostic laboratory.

The Staph-Ident system was recently introduced by Analytab Products (API), Plainview, N.Y. This commercial system requires only 5 h of incubation for the identification of staphylococci. The purpose of this study was to compare the Staph-Ident with a conventional scheme for identification of staphylococcal species isolated from bovine IMI. Additionally, the STAPHase system, a rapid tube coagulase test, was compared with the conventional tube coagulase method.

MATERIALS AND METHODS

Cultures. A total of 179 isolates, 87 from beef cow IMI and 92 from dairy cow IMI, were used. In addition, the following reference strains were obtained from the American Type Culture Collection: *S. aureus* ATCC 25923, *S. hyicus* subsp. *hyicus* ATCC 11249, *Staphylococcus capitis* ATCC 27840, *S. sciuri* subsp. *sciuri* ATCC 29062, *Staphylococcus simulans* ATCC 27848, *Staphylococcus hominis* ATCC 27844, *Staphylococcus haemolyticus ATCC 29970, Staphylococcus cohnii* ATCC 29974, *Staphylococcus warneri* ATCC 27836, *S. epidermidis* ATCC e155, *S. epidermidis* ATCC 14990, and *Staphylococcus xylosus* ATCC 29971. Jack Goldstein (Analytab Products) provided a strain of *S. intermedius*. John McDonald, National Animal Disease Laboratory, Ames, Iowa, supplied isolates of *S. hyicus* subsp. *chromogenes* NADC-A3 and *Staphylococcus saprophyticus* CCM883.

All isolates and stock cultures were plated on tryptose blood agar (Difco Laboratories, Detroit, Mich.) containing 7.5% bovine blood and 0.1% esculin (Sigma Chemical Co., St. Louis, Mo.) and incubated for 24 h at 37°C. Stock cultures were removed from tryptose blood agar plates with a sterile cotton swab, suspended in full-strength Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 20% glycerin, and stored at −20°C. All isolates were serially cultured on tryptose blood agar twice before identification. Each new lot of product and each new batch of conventional media were tested by using stock cultures as control organisms.

Conventional methodology. All biochemical tests were performed by methods described by Kloos and Schliefer for the simplified scheme (13). The Kloos and Schliefer simplified scheme was modified to identify animal species as follows: *S. intermedius*, coagulase positive, no or weak acid

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from maltose, acid from mannitol aerobically, no acid from mannitol anaerobically; *S. hyicus* subsp. *hyicus*, coagulase variable, no acid from maltose, no acid from mannitol aerobically or anaerobically, phosphatase positive, no pigment produced; *S. hyicus* subsp. *chromogenes*, coagulase negative, phosphatase positive, yellow-orange to orange pigment produced, acid from mannitol; *S. sciuri*, coagulase negative, acid from amygdalin. The phosphatase method of Devriese was used (7).

Tube coagulase tests were performed by using 0.5 ml of EDTA rabbit plasma (BBL). One colony from an 18-h tryptose blood agar culture was transferred to the plasma-containing tube, emulsified, incubated at 37°C, and observed at 4 and 24 h for clot formation.

API Staph-Ident system. The API Staph-Ident system consisted of a series of 10 microtubes containing dehydrated substrates or nutrient media (or both). The phosphatase, β-glucosidase, and β-glucuronidase tests were determined by liberation of *p*-nitrophenol from chromogenic substrates. Urea and arginine microtubes contained phenol red as the indicator. Mannitol, mannose, trehalose, and salicin microtubes contained cresol red as the indicator. The β-galactosidase (Ngp) microtube cupule required the addition of a reagent containing fast blue BB (Sigma) suspended in 2-methoxyethanol to detect free β-napthol.

Procedures recommended by the manufacturer of the Staph-Ident system were followed. Briefly, test organisms were removed from a tryptose blood agar plate with a sterile cotton swab. The swab was agitated in 2.5 ml of 0.85% saline until the turbidity produced by dispersed cells was equivalent to a no. 3 McFarland ($BaSO_4$) standard. Each microtube was inoculated with 3 drops of the bacterial suspension. Inoculated strips were placed in plastic trays and incubated for 5 h at 37°C. Color development in the Ngp microtube was recorded 30 s after the addition of the Staph-Ident reagent. Positive reactions were recorded and converted into a four-digit profile for species identification per the manufacturer's instructions. In addition to the profile register provided in each box of strips, API provided a computer printout of all possible Staph-Ident profiles. The computer printout, similar to profile indexes provided with the API 20E and API 20S, indicates first, second, and third identification choices, a frequency of occurrence for each choice, and a confidence level comment for each profile number.

STAPHase system. The STAPHase system consisted of individual microtubes containing lyophilized 2.5% EDTA rabbit plasma. Each microtube was reconstituted with 2 to 3 drops of the inoculum suspension prepared for the Staph-Ident. Strips were placed in an incubation tray, covered with a lid, and incubated at 37°C for 4 h. Loss of plasma fluidity was considered positive coagulase production.

Resolution of discrepant identifications. Adjunct testing of isolates for which both identification schemes were not in agreement were forwarded to Wesley Kloos, North Carolina State University, Raleigh, N.C., for species determination via colony morphology, pigmentation, and biochemical tests.

Phage typing. Phage typing was conducted by Fran Barnes, New York State Mastitis Control Program, Lansing, N.Y., by established methods (3, 27). All beef cow IMI isolates identified as *S. aureus* or *S. intermedius* were tested.

RESULTS

The distribution of staphylococcal species isolated from beef and dairy cow IMI is presented in Table 1. *S. aureus* was the predominant species isolated from beef and dairy cow IMI. *S. simulans* and *S. hyicus* subsp. *chromogenes* were isolated more frequently from beef cow IMI than from dairy cow IMI; however, members of the *S. epidermidis* group were more prevalent in dairy cow IMI than beef cow IMI.

TABLE 1. Identification of staphylococci from beef and dairy cow IMI

Organism	No. isolated	No. of isolates from:	
		Beef cows	Dairy cows
S. aureus	73	40	33
S. intermedius	11	6	5
S. hyicus subsp. *hyicus*	11	2	9
S. hyicus subsp. *chromogenes*	21	11	10
S. simulans	32	22	10
S. epidermidis	9	2	7
S. warneri	6	0	6
S. haemolyticus	5	0	5
S. saprophyticus	1	1	0
S. cohnii	1	0	1
S. xylosus	4	1	3
S. sciuri	2	2	0
Unclassifiable	3	0	3

Initially, the Staph-Ident system identified 89.2% of isolates (Table 2). The distribution of each species based on profile number is presented in Table 3. The Staph-Ident identified two strains of *S. aureus* as *S. xylosus*. Conventional tests determined that both strains were coagulase positive and that neither strain utilized xylose or arabinose. Only four strains of *S. hyicus* subsp. *chromogenes* were identified to the species level by the Staph-Ident system. However, by using the additional characteristic of pigment production, 13 of 21 strains were correctly identified to the subspecies level. The addition of these strains resulted in the identification of 166 isolates (94.3%). Of the remaining strains, eight unpigmented isolates were misidentified as *S. epidermidis*.

As compared to adjunct testing, 159 of 176 staphylococcal isolates (92.1%) were identified by conventional methods (Table 2). *S. aureus* strains isolated from beef cow IMI were misidentified as *S. intermedius* due to very weak maltose

TABLE 2. Comparison of conventional methods with API Staph-Ident

Organism	No. isolated	No. identified correctly	
		Conventional methods	Staph-Ident
S. aureus	73	67	73
S. intermedius	11	11	11
S. hyicus subsp. *hyicus*	11	10	10
S. hyicus subsp. *chromogenes*	21	10 (20)[a]	4 (13)[b]
S. simulans	32	32	32
S. epidermidis	9	9	9
S. warneri	6	6	6
S. haemolyticus	5	0	5
S. saprophyticus	1	1	1
S. cohnii	1	1	1
S. xylosus	4	3	4
S. sciuri	2	2	1

[a] Total number of strains correctly identified to species level.
[b] Total number of strains correctly identified to subspecies level by using yellow-orange to orange pigment as differentiating character.

TABLE 3. Frequency of Staph-Ident profile numbers among staphylococci isolated from bovine IMI

Organism	No. isolated	Profile no.	% with profile	Species sharing profile no. and differentiating characteristics[a]
S. aureus	73	7740	13.7	
		7701	2.7	*S. xylosus* (coag$^-$)
		7700	52.1	
		7500[b]	1.4	
		7300	1.4	
		6700	1.4	
		5740	2.7	
		5700	17.8	
		5500[b]	1.4	
		3700[b]	1.4	
		1700[b]	4.1	
S. intermedius	11	7741[b]	9.1	
		7541	18.2	
		7441[b]	9.1	
		5541[b]	9.1	
		5501[b]	18.2	
		3541	27.3	
		1541[b]	9.1	
S. hyicus subsp. *hyicus*	11	3540	27.3	
		3040	9.1	*S. epidermidis* (coag$^-$ mal$^+$)
		1560	9.1	
		1540	45.5	
		1520[b]	9.1	
S. hyicus subsp. *chromogenes*	21	7740[c]	4.8	*S. hyicus* subsp. *hyicus* (pig$^-$ man$^-$)
		7140	4.8	*S. epidermidis* (pig w vp$^+$)
		5540	4.8	*S. hyicus* subsp. *hyicus* (pig$^-$ man$^-$)
		3540	4.8	*S. hyicus* subsp. *hyicus* (pig$^-$ man$^-$)
		3041	4.8	*S. simulans* (phos$^{-,\pm}$ pig w)
		3040	19.0	*S. epidermidis* (pig w vp$^+$)
		3000	9.5	*S. epidermidis* (pig w vp$^+$)
		2040	4.8	*S. saprophyticus* (nov$^+$ pig w)
		1540	9.6	*S. hyicus* subsp. *hyicus* (pig$^-$ man$^-$)
		1041	9.5	*S. epidermidis* (pig w vp$^+$)
		1040	14.3	*S. epidermidis* (pig w vp$^+$)
		1000	4.8	*S. epidermidis* (pig w vp$^+$)
		0540	4.8	*S. hyicus* subsp. *hyicus* (pig$^-$ man$^-$)
S. simulans	32	6441[b]	3.1	
		3441[b]	6.3	
		3041[b]	65.6	
		3001[b]	6.3	
		2061	3.1	
		2041	15.6	
S. epidermidis	9	5040	11.1	
		3040	33.3	
		3000	11.1	
		1041[b]	22.2	
		1040	22.2	
S. warneri	6	6650[b]	16.7	
		6440[b]	50.0	
		6040[b]	16.7	
		6000[b]	16.7	
S. haemolyticus	5	4641[b]	20.0	
		4060	80.0	
S. saprophyticus	1	2040	100.0	
S. cohnii	1	0000[b]	100.00	
S. xylosus	4	7721	25.0	
		7621	25.0	
		7401	25.0	
		7021	25.0	
S. sciuri	2	5711[b]	50.0	
		1041	50.0	*S. epidermidis* (amy$^-$ xyl$^-$ ara$^-$)

[a] Abbreviations: coag, coagulase; mal, maltose; pig, pigment; man, mannitol; w, white pigment; vp, Voges-Proskaver; phos, phosphatase; nov, novobiocin; amy, amygdalin; xyl, xylose; ara, arabinose. Superscripts indicate positive or negative.

[b] Profile number not listed on sheet provided; must be obtained from computer center.

[c] *S. hyicus* subsp. *chromogenes* is not included in the data base.

utilization and non-susceptibility to phages of the international *S. aureus* bovine phage set. Adjunct testing determined that these isolates were *S. aureus*. Anaerobic mannitol utilization was added to the battery of conventional tests to discern these strains. Two strains of *S. aureus* were misidentified as *S. simulans* due to negative coagulase tests. Staph-Ident and adjunct testing determined the isolates to be *S. aureus*.

Only 10 strains of *S. hyicus* subsp. *chromogenes* were identified by conventional methods. An additional 10 strains were identified to the species level, but resolution to the subspecies level was not possible as these strains were unpigmented.

All strains of *S. simulans* and *S. epidermidis* were correctly identified by conventional methods. Three of six *S. warneri* strains were misidentified as *S. hominis* or *S. simulans* because of weak maltose utilization, inability to utilize ribose, and positive nitrate reduction tests. Five nonhemolytic, fructose-positive strains of *S. haemolyticus* were misidentified; three trehalose-negative, maltose-positive strains were identified as *S. epidermidis*. One trehalose-negative, maltose-negative, lactose-positive strain was identified as an *S. simulans* (minor route), and a weakly hemolytic, fructose-positive strain was identified as *S. warneri*. One strain of *S. xylosus* failed to utilize xylose or arabinose and was misidentified as *S. hominis*. The Staph-Ident and adjunct testing correctly delineated these isolates.

Three staphylococcal isolates were misidentified by both systems. The conventional scheme identified two isolates as *S. warneri* and one as *S. saprophyticus*; the Staph-Ident system identified the three strains as *S. saprophyticus*, *S. haemolyticus*, and *S. capitis*. Adjunct testing determined these isolates to be unclassifiable by present species descriptions.

TABLE 4. Comparison of conventional tube coagulase method with API STAPHase

Organism	No. isolated	% Positive or negative	
		Tube coagulase	STAPHase
Coagulase-positive species			
S. aureus	71[a]	100.0	98.6[b]
S. intermedius	11	100.0	90.9[b]
S. hyicus subsp. *hyicus*	5	100.0	60.0[b]
Coagulase-negative species			
S. hyicus subsp. *hyicus*	6	100.0	100.0
S. hyicus subsp. *chromogenes*	21	100.0	100.0
S. simulans	32	100.0	100.0
S. epidermidis	9	100.0	100.0
S. warneri	6	100.0	100.0
S. haemolyticus	5	100.0	100.0
S. saprophyticus	1	100.0	100.0
S. cohnii	1	100.0	100.0
S. xylosus	4	100.0	100.0
S. sciuri	2	100.0	100.0

[a] Does not include two coagulase-negative strains.
[b] All strains were coagulase positive after 24 h.

All stock cultures, with the exception of *S. epidermidis* ATCC e155, were correctly identified to the species level by both methodologies. This organism was identified by both systems as *S. warneri*.

Comparison of coagulase tests. A comparison of conventional tube coagulase and API STAPHase is presented in Table 4. Two strains of *S. aureus* were coagulase negative by both methodologies and are not considered in the final data. One strain of *S. aureus*, one strain of *S. intermedius*, and two strains of *S. hyicus* subsp. *hyicus* were STAPHase negative, but coagulase positive at 24 h by the tube method. These strains were retested and yielded a positive STAPHase reaction after 24 h of incubation. With these three exceptions, the agreement for coagulase-negative strains was 100%.

DISCUSSION

The prevalence of *S. simulans* in beef cows was surprising; however, these isolates were obtained from a research herd and may not reflect the distribution of staphylococci in beef cattle generally. The isolation of *S. epidermidis* and related organisms from dairy cows is a probable consequence of close association with humans and milking equipment contaminated with human skin flora.

S. hyicus subsp. *chromogenes* could not be distinguished from *S. epidermidis* by means of the Staph-Ident system. Use of pigment production permitted 13 of 21 strains (61.9%) to be identified, and additional characters such as acetoin production would allow delineation of nonpigmented strains. The Staph-Ident system was devised originally to permit identification of human-associated staphylococci, *S. hyicus* subsp. *hyicus* and *S. intermedius*. Consequently, the profile data base was generated with a limited number of strains representing the latter two species and none of *S. hyicus* subsp. *chromogenes* (R. Schulman, personal communication). This limits the Staph-Ident system's ability to recognize *S. hyicus* subsp. *chromogenes*. Strains of this subspecies were included in the final evaluation only after adjunct testing ascertained their identity, and pigment production was considered a reliable characteristic (8). Construction of an updated data base incorporating strains of *S. hyicus* subsp. *chromogenes* should correct this problem.

Problems encountered in the identification of staphylococci by conventional methods arose because of weak utilization or failure to utilize certain substrates, particularly *S. aureus* isolates from beef cow IMI (21). Furthermore, familiarity with each organism and the use of additional characters facilitated resolution of discrepant identifications as demonstrated by differences between conventional methods and adjunct testing.

Both systems provided acceptable levels of accuracy for the identification of staphylococci isolated from bovine IMI. However, the identification of staphylococci isolated from bovine IMI producing weak or aberrant reactions was difficult regardless of the system. Aldridge et al. (1) indicated that conventional methodologies require evaluation of each taxonomic character individually with resultant misidentifications due to aberrant reactions. These workers also determined the use of weak (±) reactions in conventional methods can cause misidentification, since interpretation of reactions is somewhat subjective and may cause confusion. Langlois et al. (15) reported that the Staph-Ident system could correctly identify 54% of staphylococci from bovine sources and indicated that many false identifications were due to incomplete color changes.

Both methods utilized in the present study were primarily developed by using biochemical characteristics of staphylococci isolated from human sources. Misidentifications encountered may be due to biotype differences of staphylococci isolated from nonhuman sources. Incorporation of biochemical characteristics generated with animal isolates into identification schemes should minimize misidentifications.

Langlois et al. (B. E. Langlois, R. J. Harmon, and K. A. Akers, Proceedings of the American Dairy Science Association Annual Meeting, p. 203, 1983) recently reported that 95% of strains isolated from bovine IMI in the University of Kentucky herd in 1969 were correctly identified by means of Staph-Ident, but ony 43% of isolates from 1981 and 1982 were correctly identified. They speculated that antibiotic therapy and postmilking teat antisepsis had reduced the prevalence of certain biotypes or species. Additionally, these workers (15) demonstrated that the Staph-Ident system identified *S. aureus* and *S. simulans* with a high degree of accuracy, but failed to delineate *S. hyicus* subsp. *chromogenes*. The results from this study agree with these findings. However, differences in sample populations may account for the accuracy level reported in each study. The University of Kentucky study had a low percentage of *S. aureus* and a high percentage of *S. hyicus* subsp. *chromogenes*, whereas the sample population in this study was approximately 41% *S. aureus* and 18% *S. simulans*.

The three unclassifiable staphylococcal isolates misidentified by both systems were similar to organisms belonging to the M group of Devriese (7).

Initially, the identification of *S. epidermidis* ATCC e155 as *S. warneri* was unexpected; however, past studies have established *S. epidermidis* ATCC e155 to be an *S. warneri* (12, 24).

A comparison of conventional tube coagulase and STAPHase methodologies demonstrated a 95% correlation for coagulase-positive staphylococci and a 100% correlation for coagulase-negative staphylococci after 4 h. However, a 100% correlation with coagulase-positive staphylococci was achieved by using a 24 h incubation period. Comparable results were achieved after 4 h with human isolates in a

previous study (9), indicating that bovine strains may produce the enzyme at a slower rate or may be encapsulated. The manufacturer has indicated that the STAPHase methodology will be changed to recommend a 24-h incubation temperature if the isolate is STAPHase negative at 4 h (R. Speziale, personal communication).

Initially, the Staph-Ident system identified 89.2% of staphylococci isolated from bovine IMI. After minor modification to permit identification *S. hyicus* subsp. *chromogenes* strains, the Staph-Ident system identifed 94.3% of isolates. An updated data base incorporating animal-associated staphylococci and availability of a complete profile index will enhance the acceptability of the product to veterinary microbiologists. Additionally, the STAPHase system was found to be an acceptable alternative to the conventional tube coagulase.

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