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Evaluation of the Minitek Gram-Positive Set for Identification of Staphylococci Isolated from the Bovine Mammary Gland

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The Minitek Gram-Positive Set was evaluated as a means of identifying staphylococci isolated from bovine mammary glands. Initial accuracy was 79.2%. Misidentification of isolates due to data base deficiencies resulted with the animal-associated species *Staphylococcus intermedius* and *S. hyicus*. Minor modification to account for data base deficiencies permitted recognition of 87.7% of the isolates. Incorporation of additional veterinary isolates into the data base would improve the accuracy of the Minitek system and enhance acceptance by veterinary microbiologists.

Staphylococcal mastitis remains a major economic problem for the dairy industry (2, 9, 18). *Staphylococcus aureus* was previously considered the only major mammary gland pathogen within the genus, whereas coagulase-negative staphylococci were considered minor pathogens (2, 9). Recent taxonomic investigations (4, 5, 7, 10, 11, 13, 14, 19–23) into the physiochemical characteristics of staphylococci have restructured the genus into 22 species. Of these, 14 have been isolated from bovine intramammary infections (1–3, 6, 16, 24). Awareness that staphylococci other than *S. aureus* cause bovine intramammary infections emphasizes the need for accurate identification of these organisms.

Kloos and Schleifer (12) developed a simplified scheme for identification of human-associated staphylococci. Subsequently, this scheme was modified to permit identification of animal-associated staphylococcal species (24). Conventional methods require numerous media, are labor intensive, and have extended incubation periods that limit their usefulness in the diagnostic laboratory.

Several commercially prepackaged systems for identification of staphylococci have been evaluated with bovine mammary gland isolates (16, 17, 24; J. L. Watts and S. C. Nickerson, Vet. Microbiol., in press). Problems encountered with these systems were due primarily to the limited number of veterinary strains incorporated into the profile data base.

Recently, BBL Microbiology Systems (Cockeysville, Md.) marketed a Gram-Positive Set for use with the Minitek identification system (MGPS). This system is designed to permit identification of staphylococci, micrococci, and streptococci. The purpose of this study was to determine the accuracy of the MGPS in identifying staphylococci isolated from bovine mammary glands.

MATERIALS AND METHODS

Cultures. A total of 130 isolates used in two previous studies (24; Watts and Nickerson, in press) were selected. All isolates were identified by a previously described conventional methodology (24). Isolates were stored in full-strength Trypticase soy broth (BBL) at −20°C until activated. Each isolate was serially cultured twice on Trypticase soy agar (BBL) supplemented with 5% bovine blood and 0.1% esculin (Sigma Chemical Co., St. Louis, Mo.). Staphylococcal beta-toxin production was determined on 5% bovine blood agar after 24 h of incubation at 37°C. Production of a wide zone of incomplete hemolysis with sharp edges was considered positive for beta-toxin production as defined in reference 8. Each new lot was tested with the following reference strains: *S. aureus* ATCC 29740, *S. hyicus* subsp. *hyicus* ATCC 11249, *S. simulans* ATCC 27848, *S. epidermidis* ATCC 14990, *S. haemolyticus* ATCC 29970, *S. hominis* ATCC 27844, *S. warneri* ATCC 27836, *S. capitis* ATCC 27840, *S. cohnii* ATCC 29974, *S. xylosus* ATCC 29971, and *S. sciuri* subsp. *sciuri* ATCC 29062. Reference strains of *S. intermedius*, *S. hyicus* subsp. *chromogenes*, and *S. saprophyticus* from a previous study (24) were also used.

MGPS. The MGPS consists of a rigid, transparent plastic plate with 20 wells into which substrate-impregnated paper disks are dispensed. The following substrate disks are included in the MGPS: arginine, arabinose, galactose, inulin, lactose, esculin, maltose, mannitol, mannose, raffinose, beta-glucosidase, salicin, sorbitol, trehalose, glucose with nitrate, Voges-Proskauer, phosphatase, hippurate, pyroglutamic acid, and leucine.

Procedures were performed as directed by the manufacturer. Briefly, test organisms were removed from a blood agar plate with a sterile cotton swab. The swab was agitated in 1.5 ml of MGPS broth until turbidity produced by dispersed cells was equivalent to a no. 1 McFarland standard. An automatic pipettor was used to dispense 0.05 ml of inoculum into each substrate well, with the exception of arginine. An inoculum volume of 0.1 ml was dispensed into the arginine well and overlaid with 0.1 ml of sterile mineral oil. Plates were covered, placed in a humidity chamber, and incubated at 37°C for 24 h. Appropriate reagents were added for determination of acetoin, nitrate reduction, hippurate hydrolysis, phosphatase, leucine aminopeptidase, and pyroglutamic acid arylamidase. Positive reactions were recorded and converted into a seven-digit profile number for species identification as directed by the manufacturer. The profile number was accessed in the MGPS profile index. The profile index provided an identification selection, confidence value, biotype validity (frequency of occurrence), supplemental tests, and atypical test results.

RESULTS

Initially, the MGPS identified 103 of 130 isolates (79.2%) correctly (Table 1). All 41 *S. aureus* strains were identified

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TABLE 1. Identification of staphylococci isolated from bovine mammary glands with the MGPS

Organism (total no. tested)	No. (%) identified correctly
S. aureus (41)	41 (100.0)
S. intermedius (4)	0[a] (0.0)
S. hyicus (40)	27[b] (67.5)
S. simulans (7)	2 (28.6)
S. epidermidis (8)	6 (75.0)
S. haemolyticus (5)	5 (100.0)
S. hominis (2)	2 (100.0)
S. warneri (10)	6 (60.0)
S. capitis (1)	1 (100.0)
S. saprophyticus (2)	2 (100.0)
S. cohnii (1)	1 (100.0)
S. xylosus (6)	6 (100.0)
S. sciuri (3)	3 (100.0)

[a] All four strains were correctly identified to the species level on the basis of beta-toxin production and mannitol utilization.

[b] Thirty-five strains were correctly identified on the basis of positive trehalose and negative acetoin production.

correctly. One mannitol-negative strain, misidentified as *S. epidermidis*, was identified correctly on the basis of coagulase production.

Four strains of *S. intermedius* included in the study were misidentified as *S. hyicus*. However, use of beta-toxin production, positive mannitol utilization, and lack of pigmentation allowed correct identification. Of 40 *S. hyicus* strains, 27 (67.5%) were identified to the species level. An additional eight strains misidentified as *S. epidermidis* were correctly identified on the basis of positive trehalose utilization and negative acetoin production. Overall, 35 of 40 *S. hyicus* strains (87.5%) were identified by the MGPS. Addition of these strains would have resulted in identification of 114 of 130 strains (87.7%).

Only two of seven *S. simulans* strains were identified by the MGPS. One strain was misidentified as *S. hyicus*, two as *S. hominis*, and one as *S. haemolyticus*. Six of eight *S. epidermidis* strains were identified correctly. One strain was misidentified as *S. hyicus* and one strain as *S. capitis*. Of 10 *S. warneri* strains, 6 were identified; 1 strain was misidentified as *S. haemolyticus*, 1 as *S. epidermidis*, and 2 as *S. hominis*. All strains of *S. hominis*, *S. haemolyticus*, *S. saprophyticus*, *S. cohnii*, *S. xylosus*, *S. sciuri*, and *S. capitis* were identified by the MGPS. All stock cultures were correctly identified to the species level except *S. intermedius*.

DISCUSSION

Previous evaluations (15–17, 24; Watts and Nickerson, in press) of commercial systems for identification of staphylococci demonstrated equal or slightly lower accuracy levels with animal isolates than human clinical isolates. Failure to recognize strains within species or omission of animal-associated species from the data base was responsible for reduced performance (24; Watts and Nickerson, in press). These studies determined that use of supplemental tests enhances the accuracy of the systems. Similar problems were encountered with the MGPS, as 79.2% of isolates were identified initially. The MGPS system did not recognize *S. intermedius* strains and misidentified strains of *S. hyicus* as *S. epidermidis*. *S. intermedius* strains were easily distinguished from *S. hyicus* strains by mannitol utilization, pigment production, and beta-toxin production, as coagulase-positive strains of *S. hyicus* subsp. *hyicus* do not produce beta-toxin or utilize mannitol (4, 6). Devriese et al. (6) reported that 57% of *S. hyicus* subsp. *chromogenes* strains utilize mannitol, but the majority of strains produce yellow-orange pigment, and all strains are coagulase negative. It was interesting that, although the species description of *S. intermedius* is referenced in the procedure brochure, no biochemical reactions or profile numbers for *S. intermedius* are listed. Omission of *S. intermedius* strains from the data base would preclude recognition of this important animal pathogen.

The majority of *S. hyicus* strains was identified correctly by the MGPS. Eight trehalose-positive, Voges-Proskauer-negative strains were misidentified as *S. epidermidis*, owing primarily to positive maltose utilization. Devriese et al. (2, 6) reported that 100% of *S. epidermidis* strains were trehalose negative and Voges-Proskauer positive and that 32 to 50% of *S. hyicus* subsp. *chromogenes* strains utilized maltose. It appears that the MGPS data base contains few strains of *S. hyicus* subsp. *chromogenes* or that the strains included were not typical.

Misidentification of *S. simulans* by the MGPS system resulted primarily from false-positive phosphatase tests. The MGPS system uses a method for phosphatase production similar to that of Kloos and Schleifer (11). Most *S. simulans* strains were reported by Kloos and Schleifer (11) as weakly phosphatase positive by the tube method, although the MGPS biochemical table lists *S. simulans* as phosphatase negative. Conventional methods (3, 5) for identifying staphylococci of bovine origin use the less sensitive disodium *para*-nitrophenylphosphate plate method to provide greater separation of weakly positive *S. simulans* strains from *S. hyicus*. Thus, misidentification of *S. simulans* may be a result of test sensitivity rather than strain differences or data base deficiencies.

Initially, the MGPS system identified 79.2% of staphylococci isolated from bovine mammary glands. Correction of data base deficiencies to accommodate recent information on biochemical activity of animal-associated staphylococcal species permitted identification of 87.7% of the isolates. Availability of an updated data base incorporating additional animal-associated staphylococcal strains would enhance the acceptability of the MGPS to veterinary microbiologists.

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