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Evaluation of the Staph-Zym System with Staphylococci Isolated from Bovine Intramammary Infections†

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A total of 148 staphylococci isolated from bovine intramammary infections were used to evaluate the Staph-Zym system (ROSCO, Taastrup, Denmark). The overall accuracy of the system was 91.9%. The system correctly identified all strains of *Staphylococcus aureus*, *Staphylococcus simulans*, and *Staphylococcus xylosus* and 95% of *Staphylococcus intermedius* strains. Of 33 *Staphylococcus hyicus* strains, 31 (93.9%) were classified correctly by the Staph-Zym system, as well as 8 (80%) of 10 *Staphylococcus chromogenes* strains. All 11 *Staphylococcus epidermidis* strains and the 1 *Staphylococcus haemolyticus* strain included in the study were identified, but the Staph-Zym system had difficulty distinguishing strains of *Staphylococcus warneri* and *Staphylococcus hominis* from other species in the *S. epidermidis* group. The Staph-Zym system correctly identified all six *S. xylosus* strains and two of three *Staphylococcus sciuri* strains. The Staph-Zym system was considered an acceptable alternative to conventional methods for identification of bovine mammary gland isolates.

Staphylococci are the organisms most frequently isolated from bovine teat skin, teat canals, and milk (2, 4, 5). Intramammary infections caused by these organisms are associated with elevated somatic cell counts and decreased milk production (1, 2, 4, 11). Furthermore, the distribution of staphylococcal species may be indicative of specific management practices (9, 10, 23). Thus, accurate identification of staphylococci is necessary for the development of improved control methods as well as for epidemiological studies.

A variety of identification methods have been used for the identification of staphylococci isolated from bovine mammary glands (16, 17, 22, 24–26). These methods include conventional methods as well as commercial identification systems. However, commercial systems may lack sufficient strains in their data bases to permit accurate identification of veterinary isolates (16, 17, 22, 24, 25). Recently, the Staph-Zym system (ROSCO, Taastrup, Denmark) became commercially available. This system is designed to identify staphylococci from both human and veterinary sources. The purpose of this study was to determine the accuracy of the Staph-Zym system in identifying staphylococci isolated from bovine intramammary infections.

MATERIALS AND METHODS

Cultures. A total of 148 staphylococcal strains was used in the study. All strains except 17 canine strains of *Staphylococcus intermedius* (obtained from H. U. Cox, Louisiana State University School of Veterinary Medicine, Baton Rouge) were isolated from bovine intramammary infections. All isolates were identified by using a previously described modification of the Kloos and Schleifer simplified scheme (20). Isolates were stored in full-strength Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 20% glycerol at −70°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.). The reference strains used in the study were *Staphylococcus aureus* ATCC 29740, *S. aureus* ATCC 25923, *Staphylococcus hyicus* ATCC 11249, *Staphylococcus chromogenes* ATCC 43764, *Staphylococcus simulans* ATCC 27848, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus warneri* ATCC 27836, *Staphylococcus hominis* ATCC 27844, *Staphylococcus haemolyticus* ATCC 29970, *Staphylococcus xylosus* ATCC 29971, and *Staphylococcus sciuri* ATCC 29062.

Conventional methodology. All biochemical tests were performed by methods described by Kloos and Schleifer for the simplified scheme (14). The Kloos and Schleifer simplified scheme was modified to identify animal species as follows: *S. intermedius*, coagulase positive, no or weak acid from maltose, acid from mannitol aerobically, no acid from mannitol anaerobically; *S. hyicus*, coagulase variable, no acid from maltose, no acid from mannitol aerobically or anaerobically, phosphatase positive, no pigment produced; *S. chromogenes*, coagulase negative, phosphatase positive, yellow-orange to orange pigment produced, acid from mannitol; and *S. sciuri*, coagulase negative, acid from cellobiose. The phosphatase method of Devriese was used (4).

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.

Staph-Zym system. The Staph-Zym system consists of a rigid, transparent plastic strip with 10 upright minitubes containing dehydrated chromogenic and modified conventional substrates (Fig. 1). Tests included in the Staph-Zym system are β-glucosidase, β-galactosidase, β-glucuronidase, trehalose, maltose, urease, arginine dihydrolase, nitrate reduction, pyrrolidonyl-aminopeptidase, and alkaline phosphatase. Additionally, susceptibilities to novobiocin (5 μg)

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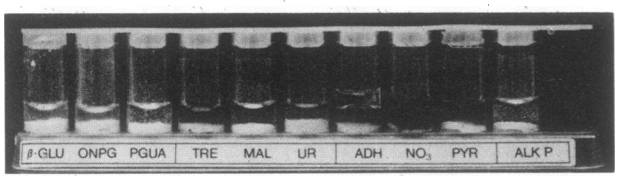

FIG. 1. Example of Staph-Zym demonstrating system format. β-Glu, β-Glucosidase; ONPG, *o*-nitrophenyl-β-D-galactopyranoside, for β-galactosidase; PGUA, β-glucuronidase; TRE, trehalose; MAL, maltose; UR, urease; ADH, arginine dihydrolase; PYR, pyrrolidonyl-aminopeptidase; ALK P, alkaline phosphatase.

and polymyxins (both antibiotics supplied as Neo-Sensitabs from ROSCO) were determined.

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 3 ml of physiological saline until turbidity produced by dispersed cells was equivalent to a no. 2 McFarland standard. Approximately 0.25 ml of the bacterial suspension was dispensed into each of the 10 minitubes, 3 drops of sterile oil was added to the arginine dihydrolase tube, and the strip was closed and incubated at 37°C for 18 to 24 h.

Prior to reading, appropriate reagents were added to the pyrrolidonyl-aminopeptidase and nitrate reduction tubes. Positive reactions were recorded and converted to four-digit numbers. A fifth digit was generated from the results of the novobiocin and polymyxins susceptibility testing. Susceptibility tests were performed on Mueller-Hinton agar by standard methods. A zone of inhibition of ≤13 mm (confluent growth) was considered resistant for both antibiotics. The five-digit profile number was accessed in the Staph-Zym profile index. The profile index provided an identification selection and supplemental tests needed for delineation of species with the same profile number.

RESULTS

Results obtained with the Staph-Zym system for 148 staphylococcal isolates are presented in Table 1. Overall, the Staph-Zym system correctly identified 136 of 148 (91.9%) isolates. All 37 strains of *S. aureus* were identified correctly. Of 20 *S. intermedius* strains, 19 were identified correctly, with 1 strain misidentified as *S. aureus*.

TABLE 1. Identification of staphylococci isolated from bovine mammary glands with the Staph-Zym system

Organism	No. (%) of isolates:	
	Tested	Identified correctly
S. aureus	37	37 (100)
S. intermedius	20[a]	19 (95)
S. hyicus	33	31[b] (93.9)
S. chromogenes	10	8[c] (80)
S. simulans	3	3 (100)
S. epidermidis	11	11 (100)
S. warneri	7	5 (71.4)
S. hominis	7	3 (42.9)
S. haemolyticus	1	1 (100)
S. xylosus	16	16 (100)
S. sciuri	3	2 (66.7)

[a] Includes 17 canine isolates.
[b] Includes three isolates identified as *S. chromogenes*.
[c] Includes four isolates identified as *S. hyicus*.

Twenty-eight of 33 *S. hyicus* isolates were identified to the species level. An additional three strains misidentified as *S. chromogenes* were identified correctly on the basis of pigment production. Four of ten *S. chromogenes* strains were identified correctly. Four strains identified as *S. hyicus* were reclassified as *S. chromogenes* on the basis of production of an orange-yellow pigment.

All three *S. simulans* strains were identified correctly, as were 11 *S. epidermidis* strains. Five of seven (71.4%) *S. warneri* strains were identified to the species level. One strain was misidentified as *S. hominis*, and one was misidentified as *S. haemolyticus*. Only three of seven (42.9%) *S. hominis* strains were identified correctly. Three strains were identified as *S. haemolyticus*, one was identified as *S. epidermidis*, and one was identified as *S. warneri*.

All six strains of *S. xylosus* were correctly identified by the Staph-Zym system. Two of three *S. sciuri* strains were classified correctly, with one strain misidentified as *Staphylococcus lentus*. All reference strains were identified correctly by the Staph-Zym system.

DISCUSSION

Accurate identification of staphylococci is important for disease control and epidemiological studies (10, 23). A commercial system should reliably identify all isolates of *S. aureus*, a primary mastitis pathogen, isolated from infected mammary glands in a dairy herd (1, 11). Furthermore, identification of coagulase-negative staphylococci is important in determining effectiveness of control measures such as postmilking teat antisepsis (9, 10, 23). As more than 40% of mammary glands may be infected with staphylococci in an individual dairy herd (23), commercial systems must also be convenient and cost-effective.

The Staph-Zym system accurately identified all strains of *S. aureus* included in the study. Furthermore, the system delineated 19 of 20 *S. intermedius* strains. *S. intermedius* is a coagulase-positive, β-toxin-producing staphylococcus isolated from carnivores (3). This organism is the predominant coagulase-positive organism isolated from dogs (3) but is infrequently isolated from bovine mastitis (21). The ability of an identification system to distinguish *S. intermedius* from *S. aureus* is important in veterinary diagnostic laboratories receiving samples from a variety of hosts. The Staph-Zym system correctly identified all 17 canine isolates in the present study. These findings concur with those of Lämmler (15), which indicated that the Staph-Zym identified 100% of 33 canine *S. intermedius* isolates. The single misidentified strain of *S. intermedius* was isolated from an infected bovine mammary gland. This strain exhibited the weak maltose utilization characteristic of *S. intermedius* but may be an atypical strain of *S. aureus* (13).

Lämmler (15) determined that the Staph-Zym system correctly identified 32 porcine and 20 bovine strains of *S. hyicus*. In the present study, the Staph-Zym system identified 9.1% of *S. hyicus* strains as *S. chromogenes* and 40% of *S. chromogenes* strains as *S. hyicus*. The primary reason for misidentification of *S. chromogenes* and *S. hyicus* strains by the Staph-Zym system was maltose utilization. *S. hyicus* is usually maltose negative by the Staph-Zym, whereas *S. chromogenes* is maltose positive. The original species description classifying *S. chromogenes* as a subspecies of *S. hyicus* (6) reported both organisms as maltose negative. However, later investigations (4, 8) determined that some strains of *S. chromogenes* are maltose positive. Cross-identification of these two species should not be considered

a major problem as these two species are easily differentiated on the basis of pigment and DNase production (4, 6, 7).

The Staph-Zym had the greatest difficulty identifying strains of *S. warneri* and *S. hominis*. These organisms were misidentified either as the other species or as *S. epidermidis* or *S. haemolyticus*. These four species and *Staphylococcus capitis* are closely related and make up a species group (12). Pulverer et al. (19) considered this group of organisms overclassified, and Namavar et al. (18) found it difficult to separate members of this group by a single phenotypic characteristic. Devriese (4) placed strains similar to *S. warneri* and *S. hominis* in a separate group termed the M group. These strains had many physiological characteristics similar to those of *S. warneri* and *S. hominis*, but the relative unreliability of some carbohydrate reactions prevented separation of these organisms into the two species (4). Similarly, cross-identification of organisms in the *S. epidermidis* group, particularly *S. hominis* and *S. warneri*, by other commercial identification systems has been reported (16, 17, 26). White et al. (27) recently reported difficulty in distinguishing between bovine strains of *S. hominis* and *S. warneri* and described a fluorogenic assay that may be useful for separation of these two species. However, the number of strains representing *S. warneri*, *S. haemolyticus*, and *S. hominis* was low; further testing of this system with additional strains is needed.

Of the novobiocin-resistant, coagulase-negative staphylococci tested, the Staph-Zym identified all *S. xylosus* strains correctly. *S. xylosus* is frequently isolated from bovine mammary glands, particularly in herds treated with chlorhexidine teat dips (10). One pyrrolidonyl-aminopeptidase-positive strain of *S. sciuri* was misidentified as *S. lentus*. These two organisms are closely related, with *S. lentus* previously classified as a subspecies of *S. sciuri* (20). No reports on pyrrolidinyl-aminopeptidase production by *S. lentus*, which is usually separated from *S. sciuri* on the basis of raffinose utilization, are available.

The Staph-Zym system costs approximately $2.80 (20 Danish kroner) per isolate, which is comparable to the cost of similar products. The Staph-Zym system offers a unique format, and is compact and convenient. Furthermore, no problems were encountered in interpreting Staph-Zym test reactions with the isolates tested. In summary, the Staph-Zym system is an accurate alternative to conventional methods for the identification of staphylococci isolated from bovine mammary glands.

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