Evaluation of the Rapid Strep System for Identification of Gram-Positive, Catalase-Negative Cocci Isolated from Bovine Intramammary Infections

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ABSTRACT

The Rapid Strep system (Analytab Products, Plainview, NY) was used to identify 199 gram-positive, catalase-negative cocci isolated from bovine intramammary infections. The system accurately identified 88.4% of isolates. The system identified 100% of 46 *Streptococcus agalactiae*, 100% of 48 *Streptococcus dysgalactiae*, 54.5% of 11 *Streptococcus equinus*, and 96.2% of 53 *Streptococcus uberis* isolates. *Enterococcus spp.* were identified correctly 83.3% of the time. One of 4 *Streptococcus saccharolyticus* strains was identified as *Streptococcus bovis*, the previous classification for this organism, and 8 *Streptococcus equi* ssp. *equi* strains were misidentified as *Streptococcus dysgalactiae*. The Rapid Strep system was determined to be an acceptable alternative to conventional methods for identification of gram-positive, catalase-negative cocci isolated from bovine intramammary infections.

INTRODUCTION

Members of the family *Streptococcaceae* are frequently isolated from bovine intramammary infections (IMI) (11, 12). This group of organisms contains both contagious and environmental pathogens (1, 12). Recent studies (2, 4, 5, 6, 7, 8, 9, 10, 15, 16) into the physiochemical characteristics of the gram-positive, catalase-negative cocci have restructured dramatically the family *Streptococcaceae*. The enterococci and lactococci are now in separate genera, *Enterococcus* and *Lactococcus* (6, 15, 16). *Streptococcus equisimilis*, group L streptococci, and the human group G streptococci now reside within the species *S. dysgalactiae* (8). Bovine and canine group G streptococci are placed in the separate species, *Streptococcus canis* (7). *Streptococcus zooepidemicus* was reclassified as a subspecies of *Streptococcus equi* (8). The group D nonenterococcus, *Streptococcus bovis*, has been incorporated into *Streptococcus equinus* (9). Additionally, two new species, *Streptococcus alactolyticus* and *Streptococcus saccharolyticus*, have been described (9).

Past methods (3, 12, 13) for the identification of mastitis streptococci did not adequately delineate newly described species. Furthermore, past methods (3, 12, 13) dependent upon conventional macrotube methods were tedious and time consuming. The Rapid Strep system (Analytab Products, Plainview, NY) is a commercial system for the 4- or 24-h identification of streptococci. Poutrel and Ryniewicz (14) reported that only 71.4% of isolates were identified by the Rapid Strep system (marketed as the API 20 STREP in Europe). These workers concluded that an improved identification key was needed to improve the accuracy of the Rapid Strep system with mastitis streptococci. However, this study was conducted before current species descriptions became available and the effect of these taxonomic changes on accuracy of the system is unknown. The purpose of this study was to evaluate the accuracy of the Rapid Strep system to identify to the species level gram-positive, catalase-negative cocci iso-

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lated from bovine intramammary infections. The ability of the system to recognize newly described species was also determined.

MATERIALS AND METHODS

Bacteria

A total of 199 gram-positive, catalase-negative cocci from a previous study were selected for use in the current study (17). A sufficient number of randomly selected strains representing each species were used in the study. A previously described scheme (17) was used for identification of all isolates. Briefly, isolates were serogrouped using the Phadebact Streptococcus Test (Pharmacia Diagnostics, Piscataway, NJ) containing groups A, B, C, and G reagents and Phadebact Group D (Pharmacia) coagglutination reagents. The following biochemical characteristics of each isolate were also determined: hydrolysis of hippurate, esculin, esculin in the presence of 40% bile, and pyroglutamate (PYR), as well as growth in 6.5% NaCl broth. Utilization of inulin, lactose, maltose, mannitol, raffinose, ribose, salicin, sorbitol, sucrose, and trehalose was also determined.

All isolates except reference strains were isolated from bovine IMI. The following reference strains were included in the study: *S. agalactiae* ATCC 27956, *S. dysgalactiae* ATCC 27957, *S. uberis* ATCC 19436, *S. uberis* ATCC 27958, *S. uberis* NCFB 2018, *S. uberis* NCFB 2038, *S. bovis* ATCC 27960, and *S. equinus* ("*S. bovis*") ATCC 33317. Prior to testing with the Rapid Strep system, all isolates were stored in 2 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD) containing 20% glycerin at −70°C. All isolates were subcultured twice on Trypticase soy agar (BBL) supplemented with 5% bovine blood and .1% esculin (Sigma Chemical Co., St. Louis, MO) before testing.

Rapid Strep System

The Rapid Strep system consists of a plastic strip with 20 cupules containing dehydrated substrates for determination of the following reactions: Voges-Proskauer, hydrolysis of hippurate and esculin, production of β-glucosidase, PYR, α-galactosidase, β-glucuronidase, β-galactosidase, alkaline phosphatase, leucine arylamidase, arginine dehydrolase, and utilization of ribose, L-arabinose, mannitol, sorbitol, lactose, trehalose, inulin, raffinose, starch, and glycogen. Hemolysis was determined on 5% bovine blood agar.

The Rapid Strep system was performed as per manufacturer's instructions with one modification. Bovine blood agar was substituted for Columbia sheep blood agar to culture test organisms prior to inoculation. An isolated colony of the test organism suspended in .3 ml of sterile distilled water was spread on an entire bovine blood agar plate. The plate was incubated anaerobically for 24 h at 35°C, and growth was removed and suspended in 2 ml of distilled water (turbidity > No. 4 McFarland standard). This suspension was used to inoculate the following tests: Voges-Proskauer, hippurate, esculin, PYR, α-galactosidase, β-glucuronidase, β-galactosidase, alkaline phosphatase, and arginine dehydrolase. The remainder of the suspension (approximately .5 ml) was added to 5 ml of Rapid Strep medium and used to inoculate the remaining tests. A blood agar plate was inoculated for hemolysis and viability determination. The arginine dehydrolase and carbohydrate utilization cupules were overlaid with sterile mineral oil to provide anaerobic conditions. The strips were placed in plastic trays, covered, and incubated for 4 h at 35°C. Reagents were added to the appropriate cupules, reactions recorded, and the generated profile number referenced in the Rapid Strep Profile Index. If the profile number was not listed, the strip was incubated for an additional 20 h as recommended. After 24 h total incubation, a new profile number was generated and the profile index referenced. All isolates yielding inconclusive results were retested.

RESULTS

Results obtained with the Rapid Strep system are presented in Table 1. Overall, the system correctly identified 88.4% of isolates. All 46 *S. agalactiae* isolates were identified correctly. Of the group C streptococci, all 48 *S. dysgalactiae* isolates were identified correctly. However, 8 *S. equi* ssp. *equi* isolates were misidentified as *S. dysgalactiae* due to positive trehalose tests.

Of 11 *S. equinus* strains tested, one was correctly identified as a *S. equinus* after 4 h of

TABLE 1. Results obtained with the Rapid Strep system for gram-positive, catalase-negative cocci isolated from bovine mammary glands.

Organism	No. tested	Identified after 4 h		Identified after 24 h	
		(no.)	(%)	(no.)	(%)
Streptococcus agalactiae	46	27	58.7	46	100.0
Streptococcus dysgalactiae	47	22	45.8	47	100.0
Streptococcus equi ssp. equi	8	0	0	0	0
Streptococcus equinus	11	1	9.1	6	54.5
Streptococcus saccharolyticus	4	0	0	1	25.0
Enterococcus spp.	30	19	63.3	25	83.3
Streptococcus uberis	53	3	5.7	51	96.2

incubation. Five strains requiring 24 h of incubation were identified as *S. bovis*. One strain was identified as *Streptococcus faecium*. One of 4 *S. saccharolyticus* strains was identified as *S. bovis*.

Twenty-five of 30 *Enterococcus faecalis* strains (83.3%) were identified as *Enterococcus* spp. Of these, 11 were classified as *E. faecalis* and 14 as *E. faecium*. Three strains were misidentified as *S. bovis* and 2 strains as *S. uberis*.

Fifty-one of 53 *S. uberis* strains tested were identified correctly by the Rapid Strep system. One hippurate-negative strain was misidentified as *S. lactis*. All reference strains were identified correctly.

DISCUSSION

Accuracy of commercial microbial identification systems is affected by the ability of inclusive tests to distinguish between species, number and diversity of strains representing each species in the database, availability of well-defined species descriptions, and accuracy of the reference scheme used for comparison. All these factors may have played a role in reduced accuracy that has been previously reported (14, 19, 20) for commercial systems with veterinary isolates. Differences in overall accuracy reported in this study and a previous study (14) may have been attributed to the aforementioned factors.

Poutrel and Ryniewicz (14) reported only 11 of 13 *S. agalactiae* strains could be identified using the Rapid Strep system. Two strains were misidentified, one due to a positive PYR test and the other due to a positive arabinose test. All 46 *S. agalactiae* strains were identified correctly by the Rapid Strep system in the present study and no strains were PYR-positive or arabinose-positive. However, a recent study reported that 34% of bovine *S. agalactiae* were PYR-positive (17) and 20% were arabinose-positive. Six strains yielding positive PYR tests by conventional methods included in the present study were PYR-negative by the Rapid Strep system. These differences in individual test results may be due to use of anaerobic incubation by the Rapid Strep system or substrate differences.

Streptococcus dysgalactiae is the most frequently isolated group C streptococci from bovine intramammary infections (12, 18). All 48 *S. dysgalactiae* strains included in the present study were identified correctly by the Rapid Strep system. Poutrel and Ryniewicz (14) encountered three ribose-negative strains that could not be identified by the Rapid Strep system and reported an overall accuracy of 81.3% for *S. dysgalactiae*. The Rapid Strep biochemical chart indicates that 100% of *S. dysgalactiae* strains are ribose-positive, whereas a recent study (17) determined that only 68.4% of bovine strains were ribose-positive after 24 h of incubation. Of the 48 *S. dysgalactiae* strains included in the present study, 12 (25.0%) were ribose-negative by conventional methods but ribose-positive by the Rapid Strep system. Differences in ribose utilization by the two systems with the same strain may be due to the metabolic state of the organism. The conventional method uses aerobic incubation, but the Rapid Strep uses anaerobic incubation.

Eight group C strains previously designated as *S. equi* ssp. *equi* were identified as *S. dysgalactiae* by the Rapid Strep system. These strains were trehalose-negative by conventional methods but were trehalose-positive by the Rapid Strep system. These may be atypical *S. dysgalactiae* strains that produce acid slowly from trehalose yielding negative results by conventional macrotube methods but positive re-

sults by the heavy inoculum, small volume rapid system.

Poutrel and Ryniewicz (14) reported that the Rapid Strep system identified only 21.4% of 14 *S. bovis* strains. Many of these strains were galactosidase-negative, a characteristic more typical of *S. equinus*. A recent taxonomic study (11) determined that *S. bovis* and *S. equinus* were related at the species level and placed both species in *S. equinus*. In the present study, 5 of 11 *S. equinus* strains were identified as *S. bovis* and one as *S. equinus*. Four additional strains were identified as *S. bovis* after retesting. Additionally, 1 of 4 *S. saccharolyticus* strains was identified as *S. bovis*, the previous classification for this organism. As both *S. equinus* and *S. saccharolyticus* are isolated frequently from bovine mammary glands (17), accurate identification is important. These results emphasize the need for an updated Rapid Strep database incorporating current classifications.

The Rapid Strep system identified 25 of 30 *E. faecalis* strains to the genus level with 11 strains identified as *E. faecalis* and 14 as *E. faecium*. Failure of these strains to utilize starch was the primary reason for misidentification as *E. faecium*. As both these enterococcal species have been isolated from bovine mastitis (18) and their distribution in the environment are similar, genus level identification for the enterococci may be adequate.

Streptococcus uberis is isolated frequently from bovine mastitis, and identification is important. Poutrel and Ryniewicz (14) reported that the Rapid Strep identified only 75% of 24 *S. uberis* strains. In the present study, 51 of 53 *S. uberis* strains (96.2%) were correctly identified by the Rapid Strep system. These differences in accuracy may be due to the accuracy of the reference system used in each study to identify the *S. uberis* strains. A recent study (17) reported that past identification methods misidentified many esculin-positive cocci as *S. uberis*. Strains included in the present study were identified using a recently described conventional method (17), which enhanced separation of *S. uberis* from similar organisms. Thus, *S. uberis* strains in the present study were a more homogeneous group than strains used in previous studies.

The Rapid Strep system permits the 4- or 24-h identification of streptococci. In the present study, only those species with unique enzymatic profiles such as *S. agalactiae*, *S. dysgalactiae*, and *Enterococcus* spp. contained a high percentage of isolates that could be identified after 4 h. The Rapid Strep system relied more heavily upon carbohydrate utilization tests for differentiation of other streptococcal species such as *S. uberis*, *S. equinus*, and *S. saccharolyticus*. As a result, 24-h incubation periods were required for a final identification.

In conclusion, the Rapid Strep system permits the rapid, accurate identification of gram-positive, catalase-negative cocci isolated from bovine intramammary infections. This system is an acceptable alternative to conventional identification schemes and has utility in veterinary diagnostic laboratories. However, the Rapid Strep database needs to be changed to reflect current classifications and the incorporation of additional veterinary strains, including newly described species, which would enhance accuracy of the system.

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