Evaluation of a Rapid System for Species Identification of Alpha-Hemolytic Streptococci

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A new 4-h commercial system (API 20S; Analytab Products, Plainview, N.Y.) for the identification of streptococci was compared with the conventional biochemical profile method in the species identification of alpha-hemolytic streptococci. A total of 194 clinical isolates (including 74 isolated from blood cultures, 64 isolated from wound cultures, and 56 isolated from respiratory cultures) and 20 reference strains were tested. Only 4 of the 20 reference strains were correctly identified to species level by the API 20S system. Six were identified to group level (viridans), four were incorrectly identified, and six did not conform to the identification key. Of the 194 clinical isolates tested, 79 (39%) were correctly identified to species level, 34 (17.5%) were identified to group level, 34 (17.5%) were incorrectly identified, and 50 (25.8%) did not conform to the identification key. Of the 12 different species of alpha-hemolytic streptococci isolated from clinical specimens, the API 20S system consistently identified Streptococcus faecalis and Streptococcus faecium, but consistently misidentified the other 10 species, especially Streptococcus mitis. Our results indicate that for identification to species level, the API 20S system is of little value for alpha-hemolytic streptococci other than enterococci.

Species identification of nonbeta-hemolytic streptococci, especially those that are alpha-hemolytic, is not usually performed by most clinical microbiology laboratories. For the most part, this group is not identified to species level because of the length of time required to obtain a complete biochemical profile; present conventional methods usually take up to 1 week. Also, most strains of alpha-hemolytic streptococci, regardless of the species, are sensitive to penicillin. (1, 7) However, alpha-hemolytic streptococci are an important cause of endocarditis and bacteremia (4, 8) and, on occasion, meningitis (5) and are being reported more often in our laboratory from these sources. In addition, we have found that certain species of alpha-hemolytic streptococci, such as Streptococcus mitis, are most often contaminants of cerebrospinal fluid rather than clinically significant organisms. Thus, species identification of streptococci may have clinical and epidemiological importance.

The development of rapid methods for species identification of alpha-hemolytic streptococci would be of great value in clinical microbiology. This not only would provide rapid results, but also would provide the clinician with more specific information on which to base initial therapeutic decisions, especially in light of a recent report of a penicillin-resistant alpha-hemolytic streptococcus (6). Recently, a new 4-h system for the identification of streptococci became available (API 20S; Analytab Products [API], Plainview, N.Y.). The purpose of this study was to compare the API 20S system with conventional biochemical methods for the species identification of alpha-hemolytic streptococci.

MATERIALS AND METHODS

The following reference cultures were obtained from the American Type Culture Collection (ATCC), (Rockville, Md.), Richard R. Facklam (Centers for Disease Control [CDC], Atlanta, Ga.), and Frank Lambert (Division of Consolidated Laboratories [DCL], Richmond, Va.): Streptococcus salivarius ATCC 9222 and CDC 262; Streptococcus mitis ATCC 9811, ATCC 9895, CDC 429, 932 (DCL), and 964 (DCL); Streptococcus faecalis ATCC 29202 and 904 (DCL); Streptococcus acidominimus CDC 1019; Aerococcus sp. ATCC 11563; Streptococcus anginosus-constellatus CDC 1111; Streptococcus MG intermedius ATCC 27335 and CDC 899; Streptococcus morbillorum CDC 1082; Streptococcus mutans CDC 909; Streptococcus sanguis I CDC 910; Streptococcus sanguis II CDC 911 and ATCC 10556; and Streptococcus uberis ATCC 9927.

A total of 194 clinical isolates were used in this evaluation. All isolates were obtained from the Clinical Microbiology Laboratories at the Medical College of

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of Virginia Hospitals, Richmond, Va. A total of 74 isolates were obtained from blood cultures, 64 from wound cultures, and 56 from respiratory cultures.

Streptococci were identified to species level by using the scheme described by Facklam (2). Biochemical tests included bile-esculin hydrolysis, arginine hydrolysis, hippurate hydrolysis, fermentation of mannitol, sorbitol, sucrose, lactose, sorbose, raffinose, arabinoose, and inulin, glucon production, growth in 6.5% NaCl, growth at 10°C, optochin sensitivity, and utilization of starch and pyruvate. Arginine hydrolysis was performed as previously described by Facklam (1). Hippurate hydrolysis and preparation and inoculation of fermentation media were performed as previously described by Hardy et al. (3). Purple broth base rather than heart infusion base (1) was used for the fermentation media. To insure that this base was adequate for performing biochemical characterizations, we used reference strains as controls with each daily run to give both positive and negative fermentation reactions. The known reference strains were correctly identified by this system. All biochemicals were held for 7 days before being reported as negative.

Streptococci were also identified by using the API 20S system. The substrates used in this system are bile-esculin, arginine, hippurate, mannitol, sorbitol, sucrose, lactose, sorbose, raffinose, glyceral, trehalose, β-glucosidase, N-acetylgalactosaminidase, β-galactosidase, indoxylacetate, phosphatase, leucine aminopeptidase, serine aminopeptidase, pyrogallactic acid arylamidase, and arginine aminopeptidase. The 4-h procedure was performed exactly as described by the manufacturer. Briefly, growth from a blood agar plate was suspended in 0.85% saline and adjusted to a final turbidity equivalent to that of a no. 1 McFarland turbidity standard. Three drops of suspension were placed into each microcapsule by using a Pasteur pipette. Strips were incubated for 4 h at 36°C without a CO₂ atmosphere. A purity plate was prepared from each inoculum to check for growth and purity of the culture and was checked after 24 h of incubation. If the inoculum did not grow (rarely) or was not pure (rarely), the initial results were discarded, and the test was repeated.

The interpretation of test results for the API 20S is similar to that for all other API systems. An octal number, generated from the reaction pattern, may lead to several possible identifications in the API profile index. In this study, we considered the API 20S system to give a species identification when the profile index stated “Viridans Streptococci (species in parenthesis)” and indicated excellent identification, very good identification, good identification, or acceptable identification. The organisms falling into these profiles were placed in the category of identification to species level. If the profile index stated “Viridans Streptococci” with no species given, and additional biochemical tests were needed, we did appropriate biochemical tests (the test then became more like a conventional system than like a rapid method), and the organisms were placed in the category of identification to group level. In addition, if the profile stated “Good likelihood, low selectivity,” additional tests were performed. The organisms in this group were also placed in the category of identification to group level. Organisms that gave a profile that did not conform to the profile index provided by API were placed in the category of not identified.

### RESULTS

A total of 20 reference cultures were identified by conventional biochemical methods and by the 4-h API 20S system. The 20 known reference strains were correctly identified by the conventional system. Subsets of these strains were run with each batch of clinical isolates to ensure reproducibility of the conventional system. Of the 20 reference strains tested in the API 20S system (Table 1), only 4 (20%) were correctly identified to species level. Six (30%) were identified to group level only, four (20%) were incorrectly identified, and six (30%) did not conform to an identification code in the API 20S data base.

A total of 194 clinical isolates were identified by both procedures (Table 2). Overall, 76

### TABLE 1. Identification of reference streptococci strains by the API 20S system

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains</th>
<th>Species level</th>
<th>Group level</th>
<th>Incorrect species</th>
<th>Not identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. anginosus-constellatus</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. salivarius</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>S. mitis</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>S. faecalis</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. acidominimus</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. MG intermedius</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. morbillorum</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>S. mutans</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>S. sanguis</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. uberis</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Aerococcus sp.</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>4 (20%)</td>
<td>6 (30%)</td>
<td>4 (20%)</td>
<td>6 (30%)</td>
</tr>
</tbody>
</table>

*Identified by conventional biochemical reactions.
The species that were identified incorrectly are listed in Table 3. There were few errors in the identification of *S. faecalis* and *S. pneumoniae*, whereas identification of *S. mitis*, *S. sanguis*, *S. salivarius*, *S. mutans*, and *S. bovis* was poor with the API 20S system. Of particular note was the observation that 11 of 16 *S. mitis* isolates were incorrectly identified as *S. pneumoniae*. In addition, *S. sanguis* II and *S. salivarius* were incorrectly identified at the species level as *S. pneumoniae* in 2 of 32 strains and 2 of 10 strains, respectively. *S. bovis*, an important cause of endocarditis, was incorrectly identified as *S. sanguis* in two of two isolates with the API 20S system.

By the end of our evaluation, API had made available an updated data base. Because of the high number of strains with a profile that did not conform to those listed in the original profile index, we ran 39 of these profiles in the updated data base. Of the 39 strains tested, 24 (61.5%) were identified to group level only, 4 (10.3%) were correctly identified to species level, 5 (12.8%) were incorrectly identified to species level, and 6 (15.4%) did not conform to an identification profile in the updated data base.

**DISCUSSION**

Species identification of alpha-hemolytic streptococci is of value in determining the identity of strains isolated from the blood of patients with recurrent endocarditis. Patients with enterococcal endocarditis require combination antimicrobial chemotherapy with a β-lactam and an aminoglycoside antimicrobial agent. Patients with endocarditis caused by viridans streptococci, in contrast, often require treatment with a single antimicrobial agent. Species identification
may aid in differentiating treatment failure from reinfection by another strain in patients with recurrent endocarditis (1). Murray et al. (7) also point out that species identification is important because *S. milleri* (*intermedius*) may be associated with serious supplicative infections. Identification and susceptibility testing may be more important in the future because of the emergence of penicillin-resistant organisms (6). We have found that species identification of alpha-hemolytic streptococci isolated from cerebrospinal fluid is useful in distinguishing clinically significant isolates from those considered to be contaminants, such as *S. mitis* (unpublished data).

Because of the advantages of identifying alpha-hemolytic streptococci, a single, rapid procedure would provide a valuable tool to the clinical microbiology service. The API 20S system, however, did not perform well in identifying the alpha-hemolytic streptococci, except for *S. faecalis* (group D). Less than half of all clinical isolates in this study were correctly identified at the species level, and almost 18% were misidentified by the API 20S system. Another 43% were not identified to species level, being identified to group level only, or were not in the API 20S data base.

The reasons for the poor performance of the API 20S system in identifying this group of organisms are unclear at this time. Because 43% of the streptococci tested in this study did not conform to an identification code in the API data base, it is obvious that the data base needs to be greatly improved. Of the biochemicals used in the API 20S profile, only nine were in common with the conventional set used in this study.

The API 20S system includes a set of enzyme substrates, used to differentiate streptococci, that are not used in the conventional scheme. Several of these substrates consistently exhibited equivocal reactions that may, in part, account for the difficulties in the system. The tests for beta-glucosidase, N-acetylglucosaminidase, beta-galactosidase, and phosphatase, as used in the API 20S system, contain chromogenic substrates, and reactions are detected by the liberation of yellow nitrophenol (ortho and para). Yellow reactions are interpreted as positive, whereas colorless to light yellow reactions are interpreted as negative. Many times, however, reactions can be interpreted as either positive or negative because the cutoff is not clearly defined. When we determined identification codes based on either positive or negative reactions, however, no difference in the final identifications was apparent.

The aminopeptidase reactions, in contrast, were more critical in determining the final identification of the alpha-hemolytic streptococci. The aminopeptidase reactions are detected by the release of beta-naphthalamines from the napthylamide substrate and produce a pink to purple color in the presence of p-dimethylaminocinnamaldehyde. In most cases, aminopeptidase reactions were clear cut; however reactions with serine aminopeptidase often gave equivocal results. Different identifications would result depending upon how the reaction was recorded.

In our opinion, further refinement of this commercial system is needed before it can replace the conventional biochemical methods for species identification of the alpha-hemolytic streptococci.

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LITERATURE CITED