Rapid Identification of Group D Streptococci with the API 20S System

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The API 20S system (Analytab Products) was evaluated as a means of identifying 209 isolates of Lancefield group D streptococci to the species level. Results were compared with those from a conventional tube biochemical identification system and from serological grouping. Use of the latest 20S computerized data base allowed species identification of 97% (203 of 209) of test isolates after a 4-h incubation period and 99% (208 of 209) of test isolates if supplemental overnight biochemical tests were used to clarify the identity of five Streptococcus bovis-variant isolates. The API 20S system appears to be a convenient and accurate method for rapid, same-day species identification of group D streptococci.

Most clinical laboratories have chosen to presumptively identify the group D streptococci and classify them as either enterococci or non-enterococcal group D streptococci based upon physiological tests such as hydrolysis of esculin in bile-esculin medium and growth in 6.5% NaCl broth (3). The relevance of this separation has been the fact that non-enterococcal group D streptococci have been found to be susceptible to penicillin, whereas enterococcal group D streptococci often require the use of combination antibiotic therapy for serious infections, usually a penicillin and an aminoglycoside (6). The majority of enterococcal group D streptococci are Streptococcus faecalis. However, 10 to 20% of enterococci isolated in hospital laboratories may be S. faecium (1). Moellerling et al. (5) have indicated that it is important to distinguish S. faecium from S. faecalis because of the fact that S. faecium isolates are more unpredictable in their susceptibility to the synergistic effects of combinations of penicillins and aminoglycosides. Therefore, it may be relevant for clinical laboratories to be able to distinguish between these species rather than simply reporting them as enterococci.

The API 20S system (Analytab Products [API]) is a recently marketed product for rapid (4-h) species identification of streptococci. We report here the results of a study in which the API 20S system was compared with a conventional tube biochemical system for species identification of group D streptococci.

MATERIALS AND METHODS

Source of test organisms. Clinical isolates of group D streptococci from the Microbial Pathology Laboratory, Medical Center Hospital, The University of Texas Health Science Center, San Antonio, comprised the majority of test organisms; in addition, 15 stock cultures (10 S. faecalis and 5 S. bovis) were used. Initial classification of isolates as group D streptococci had been achieved in the hospital laboratory by determining hydrolysis of esculin in bile-esculin medium (3).

API 20S system. Isolates were grown for 20 to 24 h on Trypticase soy agar with 5% sheep blood (BBL Microbiology Systems) and suspended in 0.85% saline (API) to achieve a turbidity equivalent to that of a no. 1 McFarland opacity standard. Each of the 20 microcups of the API 20S system was inoculated with approximately 80 µl of the standardized suspension. Inoculated API 20S strips were incubated in their accompanying humidor trays for 4 h at 37°C. Betahemolysis, which constitutes the twenty-first reaction incorporated in the profile code, was determined by examining a 24-h-old, aerobic, Trypticase soy agar- sheep blood plate.

After incubation, API reagents (ninhydrin for hippurate and cinnamaldehyde for four of the chromogenic substrates) were added to the appropriate microcups, and the reactions were interpreted exactly as described in the manufacturer's package insert. A seven-digit profile code number was generated, and the identification of each isolate was determined using the API 20S Streptococcus Analytical Profile Register. If a profile code number was not found in the Profile Register, it was submitted to API for identification with a more complete computer data base.

Reference tube biochemical identifications. Conventional macrotube biochemical test media were used as a reference method to identify each isolate. These media and the appropriate reactions for the various species have been recently described by Facklam (2). They included bile-esculin medium (Difco Laboratories), 6.5% NaCl broth (BBL), starch agar (Difco), pyruvate, arginine, hippurate, and esculin hydrolysis, and acidification of sucrose, lactose, mannitol, sorbi-
TABLE 1. Results of group D streptococcal identifications with the original 1981 and updated (May 1982) data bases

<table>
<thead>
<tr>
<th>Organism (no. tested)</th>
<th>No. (%) correctly identified with 1981 data base</th>
<th>No. (%) correctly identified with 1982 data base</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. faecalis (180)</td>
<td>180 (100)</td>
<td>180 (100)</td>
</tr>
<tr>
<td>S. faecium (8)</td>
<td>8 (100)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>S. durans (3)</td>
<td>1 (100)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>S. avium (1)</td>
<td>1 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>S. bovis (7)</td>
<td>6 (86)</td>
<td>6 (86)</td>
</tr>
<tr>
<td>S. bovis-variant (10)</td>
<td>3 (30)</td>
<td>10 (100)</td>
</tr>
</tbody>
</table>

a Total percentage, 95.
b Total percentage, 99.
c Of 10 isolates, 5 required supplemental overnight testing.

tol, arabinose, inulin, and raffinose broths prepared using heart infusion base (Difco) and brom cresol purple as the pH indicator.

Sero logical group ing. The Phadebact group D Streptococcus Test (Pharmacia Diagnostics) was used to verify that isolates were correctly categorized as members of Lancefield group D streptococci.

RESULTS

Use of the API 20S system with occasional supplemental conventional biochemical tests allowed correct species-level identification of 95% (198 of 209) of group D streptococcal isolates with the original 1981 data base and Profile Register and 99% (208 of 209) with the revised (May 1982) data base (Table 1). Use of only the API 20S system provided correct identification of 97% (203 of 209) of isolates in a 4-h period, as opposed to the 72-h incubation used with the reference tube biochemical media. The additional five isolates, which were S. bovis-variant, were correctly identified only after performance of four supplemental tests (serological grouping, fermentation of inulin and melibiose, and determination of extracellular glucans) to separate them from the second identification choice, S. salivarius. Suggestion of additional tests for separating isolates with these profile code numbers was not available in the original 1981 Profile Register. The other five S. bovis-variant isolates were correctly identified by the API 20S system without supplemental testing, and were associated with the comment "acceptable identification." Use of the updated 1982 data base significantly improved the ability to accurately identify both S. bovis-variant and S. durans (Table 1).

Reactions observed on API 20S strips with the group D streptococci were most often distinct and easily interpreted. Infrequently, degrees of color change with the nine synthetic chromogenic substrates required careful scrutiny for correct interpretation. When this occurred, it was usually restricted to the colorless, self-detecting substrates whose hydrolysis releases yellow p-nitrophenol, e.g., p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-N-acetyl-β-D-glucosamide, o-nitrophenyl-β-D-galactoside, and disodium p-nitrophénylphosphate. Faint, light-straw-colored cupules should be regarded as indicative of negative reactions. Separate observers were employed in such circumstances to verify the interpretation of weaker reactions.

As with previous API products, the probability of a profile code number representing a correct identification is determined by comparison of that profile with the typical profile for that species. This results in a comment being listed in the Profile Register along with the likelihood fraction for that profile. Thus, comments such as "excellent identification," "very good identification," or "acceptable identification" are associated with a high probability profile; comments such as "good likelihood, but low selectivity," reflect a lower level of statistical confidence. Of the 180 isolates of S. faecalis, 179 were associated with the comment excellent identification; the remaining isolate was associated with the comment good identification. Five of the eight S. faecium isolates had profile code numbers with excellent identification comments, and the other three were considered acceptable identifications. The three S. durans isolates and the S. avium isolate were associated with the comment acceptable identification. Five S. bovis isolates were regarded as good identifications, one isolate was associated with an excellent identification comment, and the seventh isolate was misidentified as S. bovis-variant, good likelihood, but low selectivity, with S. bovis listed as the third most likely identification.

Five streptococcal isolates were not readily identified by us with the conventional tube biochemical media. These included one S. faecalis, one S. faecium, one S. avium, and two S. bovis-variant. The reference identifications used for comparison with the API 20S identifications were those obtained by submitting the isolates to Richard Facklam, Centers for Disease Control, Atlanta, Ga., for arbitration. Three of these were correctly identified with the revised 1982 data base, whereas the two S. bovis-variant isolates were correctly identified only after performance of supplemental biochemical tests, as previously described.

DISCUSSION

The API 20S system is a recently marketed product for identification of members of the genus Streptococcus. It incorporates several conventional tests and a number of novel synthetic chromogenic substrates which allow identifications to be completed in only 4 h. The results of this study demonstrate that strepto-
coccii of Lancefield group D can be quickly and accurately identified with the API 20S system. The association of bacteremia caused by *S. bovis*, a non-enterococcal group D streptococcus, with neoplasms of the gastrointestinal tract, especially carcinoma of the colon (4), has stimulated interest in species identification of the group D streptococci. Thus, correct identification of *S. bovis* from blood cultures may provide the first clue to the presence of serious, but previously unrecognized, gastrointestinal disease (7). It is now recommended that any patient with *S. bovis* bacteremia undergo a thorough, aggressive evaluation of the gastrointestinal tract (4, 7).

Moellinger et al. (5) found *S. faecium* to be more resistant to synergy by combinations of penicillin and aminoglycosides than the more common enterococcal species, *S. faecalis*. They found that combinations of penicillin and streptomycin, kanamycin, tobramycin, amikacin, netilmicin, and sisomicin were unlikely to provide in vitro synergy. Only gentamicin was consistently effective in providing a synergistic effect with penicillin. Thus, it was their recommendation that enterococci should be identified to the species level if clinicians wish to use aminoglycosides other than gentamicin or streptomycin (if high-level resistance is not demonstrated) for serious infections (5).

The time required to identify streptococci with the API 20S system (4 h) allows effective pairing of the species identification of a group D streptococcus with the results of a rapid antimicrobial susceptibility test with instruments such as the MS-2 (Abbott Laboratories, Diagnostics Div.), the Autobac (General Diagnostics, Warner-Lambert Co.), or the AutoMicrobic system (Vitek Systems, Inc.). It is therefore possible to perform identification and susceptibility testing of group D streptococci with same-day methods in a manner similar to that for members of the family *Enterobacteriaceae*. The API 20S system was found, in this study, to be an accurate and convenient method of identification of this group of microorganisms.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**