Improved Recovery of Group A Beta-Hemolytic Streptococci with a New Selective Medium

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A new selective group A streptococcus agar (ssA) (BBL Microbiology Systems, Cockeysville, Md.) was evaluated for the recovery and direct plate identification of group A beta-hemolytic streptococci (GABHS) in comparison with sheep blood agar (SBA). A total of 265 GABHS were recovered from 1,116 throat swab specimens on both media, with detection rates of 98.9 and 91.7% for ssA and SBA, respectively. Primary bacitracin disk susceptibility tests were performed on 549 specimens, and presumptive identifications were possible for 81.4% of the GABHS on ssA, as compared with 44.2% on SBA. All of the 120 GABHS recovered from another 567 specimens were identified by a coagglutination method after overnight incubation on ssA, whereas only 70.2% were identified at this interval on SBA. The major advantage of the ssA was the inhibition of normal respiratory flora, permitting improved recovery and rapid identification of GABHS.

The recovery of group A beta-hemolytic streptococci (GABHS) from throat swab specimens on sheep blood agar (SBA) is a widely applied and well-accepted method. However, because of overgrowth by normal upper respiratory tract flora (NRF), detection of GABHS is often obscured, resulting in false-negative results or delays caused by labor-intensive reisolation steps. Efforts to improve the methods for GABHS recovery and identification have included supplementations of SBA with antimicrobial agents that selectively inhibit NRF (1, 4, 7–9, 11, 12, 14). The purpose of this study was to evaluate a new selective streptococcus agar (ssA) developed by BBL Microbiology Systems (Cockeysville, Md.) for the improved isolation and direct primary plate identification of GABHS.

MATERIALS AND METHODS

Specimens. A total of 1,116 oropharyngeal swab specimens were evaluated on site at the following institutions: the University of California, Davis, Medical Center, Sacramento, Calif.; The Johns Hopkins Hospital, Baltimore, Md.; and Hermann Hospital, the University of Texas Medical School, Houston, Tex.

Culture and isolation. Trypticase soy agar with 5% sheep blood (SBA) and ssA containing crystal violet, colistin, and trimethoprim-sulfamethoxazole (G. L. Evans, and T. E. O'Neil, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, C136, p. 259) was supplied by BBL. Each throat swab was inoculated in random order on both media. The inoculum was then streaked for isolation. On all of the inoculated plates, the agar was stabbed with a loop in the area of the initial inoculum and in the first zone of streaking. All plates were incubated at 35°C in a 5 to 10% CO₂ atmosphere in a CO₂ incubator. The cultures were examined after 24 and 48 h of incubation. The relative amount of NRF was noted on all primary plates examined and semiquantitated as 1+ (nonconfluent growth in the first zone of streaking only), 2+ (confluent growth in the first zone of streaking), 3+ (confluent growth in the first and second streak zones), and 4+ (confluent growth in all three streak zones).

Identification. Detection of beta-hemolytic streptococci was based on the colony morphology and beta-hemolysis on both the surface and subsurface of both media. Two methods were used for the identification of GABHS. For approximately one-half (549) of the specimens, the presumptive identification was based on bacitracin susceptibility. A differential bacitracin disk (BBL) was placed on each primary plate at the junction of the initial inoculum zone and the second zone of streaking. Presumptive identification of GABHS on the primary plate was determined only when the susceptibility to bacitracin was unequivocal. All of the beta-hemolytic streptococci that were detected were then confirmed by bacitracin susceptibility after subculture to SBA by a standard method in which a zone of inhibition of streptococcal growth is measured (5). A serological coagglutination method was used to group the beta-hemolytic streptococci recovered from 567 specimens. Five to ten isolated colonies were inoculated into 1 ml of Todd-Hewitt broth (BBL) with 1 mg of trypsin (Calbiochem-Behring, La Jolla, Calif.) per ml. The broth cultures were incubated until turbid (1 to 5 h). A drop of the broth culture was placed on a glass slide with 1 drop of each coagglutination reagent. The coagglutination reagent suspensions of Staphylococcus aureus (Cowan strain) adsorbed with rabbit antiserum to groups A, B, C, F, or G (BBL) were prepared by a standard procedure (2). Slides were rocked for approximately 30 s and observed for agglutination.

RESULTS

GABHS were recovered from 23.7% of the 1,116 oropharyngeal specimens that were tested (Table 1). Neither media recovered all 265 isolates, but ssA was significantly more sensitive, detecting 98.9% of the total, compared with 91.7% detected on SBA (P < 0.001).

ssA permitted more rapid identification of GABHS than SBA by either identification method tested (Table 2). For 549 specimens on ssA, 82.4% of the GABHS were identified by a bacitracin disk on the primary plate after overnight

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incubation, compared with only 44.2% on SBA. No false-negative or false-positive results were observed when the isolates from the direct primary plate identification were compared with the standard bacitracin disk subculture method. All of the 120 GABHS from 567 specimens that were recovered on ssA and tested by coagglutination were identified by 24 h. Only 70.2% of the GABHS recovered on SBA could be identified by this method at 24 h because of NRF overgrowth and the need for reisolation.

The recovery of non-GABHS from 567 throat cultures was determined on ssA and SBA by the coagglutination method. Group B streptococci were isolated at a similar rate on both media (27 on ssA and 24 on SBA). Seventeen beta-hemolytic streptococci of groups C, F, and G were isolated on SBA, whereas only three were detected on ssA.

A significant reduction in the presence of NRF was noted on ssA (Table 3). No growth of NRF after overnight incubation was demonstrated on 66.1% of cultures at 24 h on ssA. In addition, beta-hemolytic *Staphylococcus* spp. and *Pseudomonas aeruginosa* that were noted on SBA were inhibited on ssA.

**DISCUSSION**

The selective reduction of NRF by ssA facilitated both the recovery and identification of GABHS in this evaluation. Almost 90% of the cultures demonstrated 1+ or less NRF after overnight incubation on ssA (Table 3). NRF reduction allowed excellent visualization of GABHS during the direct examination of plated cultures. In addition, the selective activity of ssA may have enhanced the recovery of GABHS by minimizing bacterial interference that resulted from overgrowth of viridans group streptococci (13).

Identification of GABHS was more rapid by either bacitracin disk or coagglutination on ssA than on SBA. A typical positive culture on ssA appeared to be almost a pure growth of beta-hemolytic streptococci that could either be unequivocally identified by susceptibility to bacitracin or provide sufficient inoculum for coagglutination testing. Results for greater than 80% of the GABHS isolates tested by bacitracin disk and all of those tested by coagglutination were available on the same day as isolation, thus shortening the turn-around time by 1 day as compared with most routine methods. An additional advantage demonstrated by ssA was the selective reduction in the number of beta-hemolytic streptococci of groups C, F, and G. The isolation of fewer non-GABHS would not only lessen the work load required for the identification of these isolates but would decrease the number of incorrect results issued in laboratories employing the bacitracin disk for presumptive identification, since this test has been shown to yield false-positive results for non-GABHS (6).

The issues that have limited the use of selective media for the isolation of GABHS, particularly media containing trimethoprim-sulfamethoxazole, have included high false-negative rates (3) and delayed growth (10). These observations, however, may reflect a medium-dependent phenomenon. For example, most GABHS will not grow on thymidine-free media in the presence of trimethoprim-sulfamethoxazole (8). However, GABHS are better able than NRF to utilize the thymidine present in tryptic soy agar and thus circumvent the metabolic inhibition of trimethoprim-sulfamethoxazole. Therefore, the preparation and performance of selective media containing this antimicrobial agent must have excellent quality control. In the present evaluation of ssA, the manufacturer has met these criteria and produced a medium that demonstrated a low false-negative rate (1.1, versus 8.2% for SBA) and excellent growth of GABHS after overnight incubation. In fact, of the 262 GABHS detected on ssA, 244 were observed after overnight incubation. Four lots of ssA were used in this evaluation, with no noted lot-to-lot variation.

These results indicate that ssA is a very useful selective culture medium that offers significant advantages over SBA for the primary isolation of GABHS from throat swab specimens. The selective suppression of NRF on ssA facilitated the interpretation of cultures with enhanced recovery and rapid identification of GABHS.

**LITERATURE CITED**


SELECTIVE ISOLATION OF GROUP A STREPTOCOCCI


