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Effects of Trimethoprim-Sulfamethoxazole and Incubation Atmosphere on Isolation of Group A Streptococci

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The effects of selective media and incubation atmosphere on the isolation of group A beta-hemolytic streptococci were evaluated. A higher percentage of group A streptococci was isolated on sheep blood agar incubated in air than in CO_2 or anaerobic atmospheric conditions. Fewer non-group A beta-hemolytic streptococci were isolated on sheep blood agar incubated in air than in CO_2 or anaerobically. Group A streptococcal isolation was not significantly affected by different incubation atmospheres on sheep blood agar containing trimethoprim-sulfamethoxazole, but detection time was longer than on sheep blood agar alone. No significant difference was found between isolation of group A streptococci on sheep blood agar incubated in air and that on sheep blood agar containing trimethoprim-sulfamethoxazole and incubated in 5 to 10% CO_2; however, more group A streptococci were isolated on sheep blood agar in air within 24 h. Sheep blood agar incubated at 35°C in air is, therefore, recommended for the isolation of group A streptococci from throat swabs.

The diagnosis of group A streptococcal pharyngitis is still, for all practical purposes, based on isolation of the organism from throat cultures on agar containing approximately 5% sheep blood (SBA). Efforts to improve the isolation of group A streptococci have included supplementation of SBA with various antimicrobial agents (1–6, 10, 13) and incubation of the media under different atmospheres (3, 7, 8, 10). Most recently, the combination of sulfamethoxazole and trimethoprim has been added to sheep blood agar (SBA/SXT) to increase the sensitivity of detection of group A streptococci (2, 4–6). The purpose of this study was to evaluate the effects of incubation atmosphere on the isolation of group A streptococci from throat cultures inoculated onto SBA and SBA/SXT.

MATERIALS AND METHODS

Oropharyngeal specimens were collected with Dacron swabs (Culturette; Marion Scientific Corp.). The isolation of group A streptococci on selective media was evaluated by inoculating each clinically submitted throat swab specimen in random order onto each of three plates of tryptic soy agar (GIBCO Laboratories) containing 5% sheep blood (SBA) and onto three SBA plates supplemented with 23.75 μg of trimethoprim and 1.25 μg of sulfamethoxazole per ml (SBA/SXT). The throat swab specimen was rolled across one-fourth of each plate, and the inoculum was then streaked with a wire loop onto the remaining quadrants. On all inoculated plates, a few stabs into the agar were made with the loop in the area of initial inoculum and in the areas of the isolation streak. One SBA/SXT plate was then incubated at 35°C in air, in 5 to 10% CO_2 in a CO_2 incubator, and anaerobically in an anaerobic chamber containing an atmosphere of 85% N_2, 10% H_2, and 5% CO_2. After 24 and 48 h, each plate was examined independently by two separate observers for the presence or absence of beta-hemolytic streptococci. Examination after 24 h of plates incubated in CO_2 and anaerobic conditions required 15 to 30 min of exposure to room air. Group A streptococci were identified by the direct fluorescent antibody technique (9). Data were analyzed by chi-square analysis or by student's t test as appropriate.

To examine the possibility of a dilutional effect resulting from the inoculation of each swab onto six agar media, preliminary experiments were performed in which a series of patient swabs which had been stored for 16 to 24 h at 5°C and which had yielded group A streptococci were each inoculated consecutively onto each of six SBA plates. Examination of these cultures after 18 h of incubation demonstrated no differences between the first and last plates as regards the number of beta-hemolytic colonies, their morphology, or the extent of hemolysis surrounding them.

RESULTS

The isolation of group A streptococci from SBA and SBA/SXT under three different incubation atmospheres is noted in Table 1. No group A streptococci were detected only as the result of subsurface hemolytic reactions in the stabbed areas of the agar. Of 1,805 throat swabs cultured, group A streptococci were recovered from 18.1% on SBA-air, 15.6% on SBA-CO_2,

TABLE 1. Effect of atmosphere and duration of incubation on recovery of group A streptococci from SBA and SBA/SXT

Medium-atmosphere	No. (%) of cultures positive for group A streptococci	
	24 h	48 h[a]
SBA-air	294 (16.3)	327 (18.1)
SBA-CO_2	209 (11.6)	282 (15.6)
SBA-anaerobic	236 (13.1)	309 (17.1)
SBA/SXT-air	101 (10.6)	312 (17.3)
SBA/SXT-CO_2	211 (11.7)	321 (17.8)
SBA/SXT-anaerobic	224 (12.4)	309 (17.1)

[a] Cumulative number (percent) after 48 h of incubation.

and 17.1% on SBA-anaerobic after 48 h of incubation. More group A streptococci were isolated on SBA-air than an SBA-CO_2 ($p < 0.01$) or SBA-anaerobic ($p < 0.05$) within 24 and 48 h. Different incubation atmospheres did not significantly affect group A streptococcal isolation from SBA/SXT at 48 h (Table 1). However, comparison of the isolation rates on the two media showed the detection time to be longer on SBA/SXT than on SBA.

The effects of incubation in air, 5 to 10% CO_2, or anaerobically on the isolation of beta-hemolytic streptococci were analyzed after 24 and 48 h of incubation. Beta-hemolytic streptococci were recovered significantly less frequently with incubation in air than with incubation in CO_2 or anaerobically. This difference was due to the recovery of fewer non-group A beta-hemolytic streptococci on plates incubated in air compared to those incubated in CO_2 and anaerobically. The comparison of both media and incubation atmospheres on the isolation of non-group A streptococci is shown in Table 2. More non-group A streptococci were isolated on SBA-anaerobic (29.4%) and SBA-CO_2 (13.3%) than on SBA-air (11.9%). The degree of beta-hemolysis (as measured by the zone of hemolysis) was less on SBA/SXT than on SBA, and no significant difference in isolation of non-group A beta-hemolytic streptococci existed among different incubation atmospheres on SBA/SXT media.

In comparing pairs of media by incubation atmosphere, group A streptococci were isolated more frequently on SBA-air (18.1%) than on SBA/SXT (17.3%; $p < 0.05$) and more frequently on SBA/SXT-CO_2 (17.8%) than on SBA-CO_2 (15.6%; $p < 0.05$). No significant difference was found between SBA-anaerobic and SBA/SXT-anaerobic (17.1%). At 48 h, no statistically significant difference was found between SBA-air and SBA/SXT-CO_2, but more group A streptococci were isolated on SBA-air (16.3%) than on SBA/SXT-CO_2 (11.7%; $p < 0.05$) within 24 h.

DISCUSSION

Recovery of group A streptococci from throat swab specimens is influenced by many factors, such as the method of acquiring the culture, transportation media used, and the laboratory methodology. Incubation of cultures in atmospheres consisting of air, air with added CO_2, and under anaerobic conditions have each been recommended for isolation of group A streptococci. In this study of 1,805 throat culture specimens, a higher percentage of group A streptococci was isolated on SBA incubated in air than on SBA incubated in CO_2 or anaerobically. The growth of non-group A beta-hemolytic streptococci was increased in anaerobic incubation conditions and less so in air plus CO_2 (Table 2). Consequently, our results demonstrated that growth on SBA in an atmosphere of air was the most sensitive and specific means of detecting group A streptococci.

In an effort to decrease the overgrowth by other aerobic and facultatively anaerobic oropharyngeal bacteria in CO_2 and anaerobic incubation conditions (11), several investigators have evaluated the use of antibiotic-supplemented media with conflicting results. Vincent et al. (13) reported that group A streptococci were isolated as frequently on antibiotic-free SBA as on SBA supplemented with neomycin and nalidixic acid. Murray et al. (10) found that SBA supplemented with 2.5 or 5.0 μg of gentamicin per ml was less successful in isolating group A streptococci than antibiotic-free media. More recently, Kurzynski et al. (5, 6) reported that group A streptococci were more frequently isolated on SBA/SXT than with unsupplemented SBA plates when both were incubated in CO_2. In contrast, Dykstra et al. (2) found SBA plates incubated anaerobically to be superior to SBA/SXT which was incubated in both CO_2 and anaerobic atmospheres. The reasons for these differences in results are not readily apparent.

Since overgrowth of group A streptococci by other aerobic and facultatively anaerobic bacteria was enhanced in the presence of 5 to 10%

TABLE 2. Effect of media and incubation atmosphere on isolation of non-group A beta-hemolytic streptococci

Medium-atmosphere	No. (%) of cultures positive for non-group A beta-hemolytic streptococci
SBA-air	215 (11.9)
SBA-CO_2	240 (13.3)
SBA-anaerobic	530 (29.4)
SBA/SXT-air	130 (7.2)
SBA/SXT-CO_2	145 (8.0)
SBA/SXT-anaerobic	164 (9.1)

CO_2, it would appear that the incorporation of trimethoprim and sulfamethoxazole is of greater benefit under these conditions than when the media are incubated in an atmosphere of room air. Since the combination of trimethoprim and sulfamethoxazole is generally ineffective against anaerobic bacteria (12), it appears that the combination is not helpful in preventing overgrowth of group A streptococci under anaerobic incubation conditions.

In conclusion, SBA/SXT significantly decreased beta-hemolysis and delayed detection of group A streptococci. The optimal method for detection of group A streptococci within 24 h of incubation was culture on nonselective SBA and incubation at 35°C in air. Incubation for 48 h increased the number of cultures positive for group A streptococci on both media and under each condition of incubation; however, the yield after this period remained higher for throat swabs cultured on nonselective SBA which was incubated in air.

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