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Effects of Selective Media and Atmosphere of Incubation on the Isolation of Group A Streptococci

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The effects of selective media and atmosphere of incubation on the isolation of group A beta-hemolytic streptococci were evaluated. Sheep blood agar medium with gentamicin (2.5 or 5.0 μg/ml) was inferior to antibiotic-free sheep blood agar medium. This resulted from the partial restriction of group A streptococcal growth on gentamicin medium. Recovery of beta-hemolytic streptococci from specimens in air, CO_2, or anaerobic incubation was evaluated. The isolation of group A streptococci was equivalent in the three incubation atmospheres; however, non-group A beta-hemolytic streptococci were isolated significantly more often from specimens incubated in an anaerobic or CO_2 atmosphere than from those incubated in air. Therefore, sheep blood agar medium, stabbed with a wire loop and incubated in air, is recommended for the isolation of group A streptococci from throat swabs.

The accurate detection of group A beta-hemolytic streptococcal pharyngitis is important to prevent its potential sequelae, rheumatic fever and acute glomerulonephritis (8). Because the clinical diagnosis of streptococcal pharyngitis is unreliable (4, 10), it is necessary to isolate and identify the etiological agent. Several techniques have been proposed to increase the recovery of beta-hemolytic streptococci, including the incorporation of antibiotics into the blood agar medium (1-3, 14), incubation in the presence of CO_2 (6), and incubation in the absence of oxygen (9). Incubation in a CO_2 atmosphere increases the recovery of the more fastidious streptococci, whereas beta-hemolysis is optimally detected with anaerobic incubation.

In this study, the effects of selective media and the atmosphere of incubation on the isolation of group A streptococci from throat swab specimens were evaluated.

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 throat swab specimen in random order onto a plate of Trypticase soy agar (GIBCO) supplemented with 5% sheep blood (SBA) and onto a SBA plate with added gentamicin in concentrations of either 2.5 or 5.0 μg/ml. The composition of the commercially prepared SBA and the SBA-gentamicin plates prepared in this laboratory was the same except for the added antibiotic. In both plates of media, 5% antibiotic-free sheep blood (GIBCO), with an average hematocrit of 30%, was used. The throat swab specimen was rolled across one-fourth of the plate, and the inoculum was then streaked with a sterile wire loop into 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 streaks. The plates were then incubated at 35°C in an atmosphere of 3 to 5% CO_2. The isolation of group A streptococci from specimens incubated in different atmospheric conditions was evaluated by inoculating each specimen in random order onto three commercially prepared SBA plates as described above. The plates were then incubated at 35°C in air, CO_2, or an anaerobic glovebox chamber with an atmosphere of 85% N_2, 10% H_2, and 5% CO_2. After overnight incubation, the plates were examined for the presence or absence of beta-hemolytic streptococci. Group A beta-hemolytic streptococci were identified by the fluorescent antibody technique (11). We have also included data obtained after incubation for 48 h because, in our experience, some organisms and many SBA plates have demonstrated detectable hemolysis only after prolonged incubation.

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RESULTS

Comparison of SBA and SBA-gentamicin media. The isolation of group A streptococci from 443 throat swab specimens was compared on SBA and on SBA with gentamicin (5.0 μg/ml; Table 1). Of 65 specimens with group A streptococci, 63 (97%) were isolated on SBA medium and only 30 (46%) were isolated on SBA-gentamicin medium. The isolation of

TABLE 1. *Number of isolates of beta-hemolytic streptococci on SBA and on SBA-gentamicin (5.0 μg/ml)*

Determination	No. of isolates	
	SBA	SBA-gentamicin (5.0 μg/ml)
Throat specimens	443	443
Beta-hemolytic streptococci	118	89
Streptococci, group A[a]	63	30
Streptococci, not group A	55	59

[a] A total of 65 group A streptococci were isolated.

group A streptococci from an additional 587 specimens was compared on SBA and on SBA with gentamicin (2.5 μg/ml; Table 2). Of 105 specimens with group A streptococci, 96 (91%) were isolated on SBA medium and 80 (76%) were isolated on SBA-gentamicin medium.

Comparison of incubation atmospheres. The effects of incubation in air, 3 to 5% CO_2, or an anaerobic chamber on the isolation of beta-hemolytic streptococci were analyzed (Table 3). Because each specimen was inoculated onto three plates, which were then randomly incubated in the three atmospheres, the recovery of beta-hemolytic streptococci on the first inoculated plate was examined to approximate the routine laboratory situation. After both 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 anaerobic incubation. This resulted from the recovery of fewer non-group A streptococci on plates incubated in air than on plates incubated in CO_2 or anaerobiosis (chi-square test, $P < 0.001$). Group A streptococci were recovered with equal frequency from plates incubated in any of the three atmospheres after 24 and 48 h of incubation (Table 3). In addition, the percentage of group A streptococci recovered on the first of three plates inoculated was equivalent in all three atmospheres (Table 4).

DISCUSSION

The recovery of group A streptococci from throat swab specimens is influenced by the extent of pharyngeal involvement, the method used in swabbing the throat, the laboratory methods, and the presence of bacteria indigenous to the mouth and inhibitory to group A streptococci. In two studies of patients with streptococcal pharyngitis, 73 and 92% of the group A streptococci were detected with a single throat swab (4, 7). Saslaw and co-workers (13) found a large variation in the number of group A streptococci recovered from throat swab specimens from patients with streptococcal infections. Increased recovery of group A streptococci by the selective inhibition of normal oropharyngeal bacteria with antibiotic-supplemented media has been attempted by several investigators. Vincent and co-workers (14) reported, however, that group A streptococci were isolated as frequently on antibiotic-free SBA as on SBA supplemented with neomycin and nalidixic acid. Black and Van Buskirk (1, 2) found that, although 25% of the beta-hemolytic streptococci isolated from non-oro-

TABLE 2. *Number of isolates of beta-hemolytic streptococci on SBA and on SBA-gentamicin (2.5 μg/ml)*

Determination	No. of isolates	
	SBA	SBA-gentamicin (2.5 μg/ml)
Throat specimens	587	587
Beta-hemolytic streptococci	173	139
Streptococci, group A[a]	96	80
Streptococci, not group A	77	59

[a] A total of 105 group A streptococci were isolated.

TABLE 3. *Effects of atmosphere and duration of incubation on recovery of beta-hemolytic streptococci*

Incubation atmosphere	Incubation time (h)	No. of specimens	No. of isolates		% of specimens	
			Non-group A	Group A	With non-group A	With group A
Air	24	264	22	56	8.3	21.2
CO_2	24	349	47	68	13.5	19.5
Anaerobic	24	255	64	60	25.1	23.5
Air	48	264	25	59	9.5	22.3
CO_2	48	349	52	74	14.9	21.2
Anaerobic	48	255	73	61	28.6	23.9

TABLE 4. *Number of isolates of group A streptococci on the first inoculated plate*

Incubation atmosphere	Incubation time (h)	No. of isolates		% Isolated on first plate
		On first plate	On second or third plates only	
Air	24	56	5	91.8
CO_2	24	68	10	87.2
Anaerobic	24	60	4	93.8
Air	48	59	3	95.2
CO_2	48	74	4	94.9
Anaerobic	48	61	3	95.3

pharyngeal sources were recovered only on SBA with gentamicin, the recovery from throat specimens with this medium was no better than with antibiotic-free SBA. In our study, fewer group A streptococci were recovered on SBA with gentamicin than on antibiotic-free SBA. This result was unexpected because, although gentamicin is active against staphylococci and gram-negative bacilli, streptococci are relatively resistant to gentamicin. The streptococci isolated only on the SBA medium grew on the SBA-gentamicin medium on subculture, but the colony size and zone of hemolysis on SBA-gentamicin medium were smaller than on the antibiotic-free medium. The inability of gentamicin, therefore, to inhibit the normal oropharyngeal bacteria (including the viridans streptococci) inhibitory for group A streptococci (5, 12), and the reduced growth and hemolysis of the group A streptococci, would account for the unsatisfactory results with the SBA-gentamicin medium.

Air, CO_2, or anaerobic incubation has been recommended for the isolation of group A streptococci; however, a comparative study with an adequate number of specimens has never been performed. Although the colonial morphology of the streptococci and the frequency of alpha prime hemolysis were similar in the different atmospheres, the degree of beta-hemolysis was greatest in anaerobic incubation. This resulted from the activity of oxygen-unstable streptolysin O. In this study, the maximal recovery of beta-hemolytic streptococci was with anaerobic incubation (Table 3); however, the majority of these beta-hemolytic streptococci were not group A. Non-group A streptococci were isolated from 8 to 10% of the specimens incubated in air; in contrast, non-group A streptococci were isolated from 13 to 15% and 25 to 29% of the specimens incubated in CO_2 and anaerobiosis, respectively. Although the isolation of non-group A streptococci was variable for the three incubation atmospheres, the recovery of group A streptococci was not statistically different for the three atmospheres after both 24 and 48 h of incubation. Group A streptococci were isolated from a similar percentage of specimens, between 21 and 24% (Table 3), and with equal frequency on the first inoculated plate (95%; Table 4) in the three different atmospheres after 48 h of incubation.

In summary, significantly more non-group A streptococci were isolated in anaerobic and CO_2 incubation than in air incubation. Since the greater incubation and identification costs of anaerobic and CO_2 incubation are not justified by an increase in group A streptococcal detection, the routine incubation of specimens in air is recommended. In addition, because of the poor recovery of group A streptococci on SBA-gentamicin plates, the use of antibiotic-free SBA plates is recommended.

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