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Pharyngeal Carriage of Group B Streptococci: Detection by Three Methods

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The prevalence of pharyngeal carriage of group B streptococci was evaluated in patients with and without the complaint of a sore throat by three methods (blood agar plates, Columbia CNA agar plates, and a selective enrichment broth containing gentamicin and nalidixic acid). The overall carriage rate of group B streptococci was 12%, and there was no significant difference between the two groups of patients. The selective broth medium was more sensitive than the two solid agar plate methods in detecting carriage, and 37% of all group B streptococci were recovered solely from the broth. Use of the broth alone would have permitted a 94% detection of the group B streptococcal carriers.

The few published studies of the isolation of *Streptococcus agalactiae* or group B streptococcus from the throat have consistently reported relatively low rates of carriage. In an early study limited to a small number of nurses and school boys in England, Hare reported a carriage rate of 5% (6). Another investigation of both healthy soldiers and those with respiratory infections revealed 0.1 and 0.2% carriage rates, respectively (3). None of these studies, however, focused specifically on group B streptococci nor were special efforts taken to optimize the isolation of these organisms. More recent studies have revealed that ca. 5% of pregnant women and nursery personnel have positive throat cultures for group B streptococci (1, 5).

Although group B streptococci have been recovered from patients with clinical pharyngitis (7), the significance of this finding is unclear, since group B streptococci have not been regarded as pharyngeal pathogens (12). However, group B streptococci are commonly isolated from the blood, cerebrospinal fluid, urine, wounds, and other sites or tissues of patients with clinical disease (12). In addition, ample epidemiological evidence has accumulated to support the theory that the maternal genital tract transmits group B streptococci to newborn infants and that these organisms play a role in causing neonatal sepsis and meningitis (1, 4, 5, 12).

This study was undertaken to investigate whether the throat is an important reservoir of group B streptococci, to determine the carriage rates in young adults with and without upper respiratory symptoms, and to evaluate the use of a selective enrichment broth for recovery of group B streptococci. A selective broth has been shown to enhance the isolation of group B streptococci from genital tract cultures and to identify infants contaminated with these bacteria in the newborn period (2, 9).

It has been theorized and demonstrated that several thousand streptococci must be present in a specimen before they can be recovered on ordinary media by direct plating (11). The success of recovery also depends on the proportion of streptococci to other organisms in the specimen. Thus, the advantage of using a selective enrichment medium that would increase the sensitivity of recovery of group B streptococci by inhibiting the growth of most normal flora becomes apparent.

MATERIALS AND METHODS

All cultures were obtained from patients at the Boynton Student Health Service, University of Minnesota, Minneapolis. Test patients were selected on the basis of their complaint of a sore throat and control patients were selected on the basis of their nonrespiratory complaints. An age limitation of 16 to 32 years was imposed to restrict the study to young adults frequenting the clinic. The purpose of the study was explained to the patients, and they were given the opportunity to volunteer.

Pharyngeal cultures were obtained in triplicate by using a rayon-tipped swab (Culturette, Marion Scientific Corporation, Rockford, Ill., with modified Stuart transport medium). Swabs were refrigerated until plated, with an average of 2.5 h between collection and plating.

Culture media. (i) Selective broth medium. The broth described by Baker et al. (2) was adapted for use. This consisted of Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.), 5% sheep blood, 8 μg of

gentamicin sulfate (Schering Laboratories, Bloomfield, N.J.) per ml, and 15 μg of nalidixic acid (Sigma Chemical Co., St. Louis, Mo.) per ml.

(ii) **Solid media.** Tryptose blood agar base (Difco) with 6% sheep blood and Columbia CNA agar (Difco) containing 10 μg of colistin per ml and 15 μg of nalidixic acid per ml with 6% sheep blood were used as primary plating media.

Processing of cultures. One swab was inoculated onto a sheep blood agar plate, a second was inoculated onto a selective (CNA) blood agar plate, and a third was placed in the selective broth. All plates were incubated aerobically at 35°C for 18 h overnight and then at 25°C for 24 h before being discarded as negative for β-hemolytic streptococci. The broth was incubated at 35°C for 48 h and then streaked onto a sheep blood agar plate. All suspicious β-hemolytic colonies were subcultured for further identification, and a Gram stain was performed when necessary to confirm the presence of a gram-positive coccus. Special attention was given to examining all plates for nonhemolytic colonies morphologically resembling streptococci; these organisms were also studied further.

Streptococcal grouping. Presumptive identification of group A streptococci was performed by using bacitracin disks (Baltimore Biological Laboratory, Cockeysville, Md.) (10). Definitive serological grouping of all streptococci was performed by the hot-acid extract capillary precipitin method of Lancefield (8), with rabbit antisera prepared in our laboratory or provided by the Center for Disease Control, Atlanta, Ga.

Statistical methods. Differences were statistically analyzed by the chi-square test, with the Yates correction for small numbers, and by the standard error of the difference between two proportions.

RESULTS

During a 7-week period, throat cultures were obtained from 408 individuals, 258 of whom, complaining of a sore throat, were in the test group. The remaining 150 comprised the control group. There was little variation between the age or sex distribution in the two study groups. There were 208 males (51%) and 200 females (49%). The mean age was 23 years, with test individuals averaging 1 year more than the control patients.

Nearly 25% of the entire study population had positive throat cultures for β-hemolytic streptococci (Table 1). The carriage rate was 25% for the test group and 19% for the control group. The overall prevalence of group B streptococcal carriage was 12%; in patients with sore throats, it was nearly 14%; and in those patients with nonrespiratory symptoms, it was 9% ($P > 0.10$).

Males in both study groups showed little variation in overall β-hemolytic streptococcal and group B streptococcal carriage rates (Table 2). Females in the test group were found to carry

TABLE 1. *Prevalence of β-hemolytic streptococci*

Study group	No. of individuals cultured	No.[a] carrying: β-Hemolytic streptococci	No.[a] carrying: Group B streptococci
Test	258	65 (25.2)	35 (13.6)
Control	150	28 (18.7)	14 (9.3)
Total	408	93 (22.8)	49 (12.0)

[a] Number positive by one or more of the three methods used. Numbers in parentheses indicate percentages.

TABLE 2. *Comparison of streptococcal carriage rates by sex*

Study group	No. cultured	No. carrying[a]: β-Hemolytic streptococci	No. carrying[a]: Group B streptococci
Test[b]			
Male	137	33 (24.1)	17 (12.4)
Female	121	32 (26.4)	18 (14.9)
Control[c]			
Male	71	17 (23.9)	8 (11.3)
Female	79	11 (13.9)	6 (7.6)

[a] Numbers in parentheses indicate percentages.
[b] Total, 258.
[c] Total, 150.

both β-hemolytic streptococci and group B streptococci approximately twice as frequently as those in the control group (Table 2). The differences in group B streptococcal carriage between women in the two groups were not significant ($P > 0.10$) nor were the differences in overall streptococcal carriage ($0.10 > P > 0.05$).

All of the streptococcal strains were groupable (Table 3), the most prevalent group being group B (52%), followed by groups F (22%) and G (11%). Two patients harbored two different strains of β-hemolytic streptococci in the throat. One had both groups B and F, and the other had groups B and C. The group A streptococci, as determined by the definitive precipitin method, were all bacitracin positive. There were five (10%) bacitracin-positive group B streptococci and one group G bacitracin-positive streptococcus. No nonhemolytic strains of group B streptococci were detected.

The selective broth had the highest recovery rate of group B streptococci (Table 4). Of the 49 patients from whom group B streptococci were isolated, 18 (37%) were identified by the broth method only. Similar percentages of patients in both groups were detected by this method alone. Thirteen patients (27%) were identified as carriers of group B streptococci by all three methods. A combination of blood agar plates

TABLE 3. *Distribution of streptococcal strains by serological group*

Study group	No. of strains	No. of strains recovered of group[a]:				
		A	B	C	F	G
Test	67[b]	7 (10.4)	35 (52.2)	3 (4.5)	13 (19.4)	9 (13.5)
Control	28[c]	2 (7.1)	14 (50.0)	3 (10.7)	8 (28.6)	1 (3.6)
Total	95	9 (9.4)	49 (51.6)	6 (6.3)	21 (22.1)	10 (10.6)

[a] Numbers in parentheses represent percentages.
[b] Percentage is based on 67 strains isolated from 65 culture-positive patients; one patient had both groups B and F, and one had groups B and C in the throat.
[c] Isolated from 28 culture-positive patients.

TABLE 4. *Number of group B streptococcal carriers detected by three methods*

Method of detection	No. of patients identified as positive for group B streptococci[a]	
	Test group	Control group
SBA[b] only	1 (2.9)	1 (7.2)
CNA[c] only	0 (0)	0 (0)
SEB[d] only	13 (37.1)	5 (35.7)
SBA and CNA	1 (2.9)	0 (0)
CNA and SEB	6 (17.1)	0 (0)
SBA and SEB	6 (17.1)	3 (21.4)
SBA, CNA, and SEB	8 (22.9)	5 (35.7)
Total	35	14

[a] Numbers in parentheses represent percentages.
[b] Sheep blood agar plate.
[c] Columbia CNA agar plate.
[d] Selective enrichment broth.

and broth detected nine individuals (18%) as positive for group B streptococci, and both the CNA agar plates and the selective broth method identified six patients as positive. Blood agar and selective (CNA) agar plates were less sensitive methods for detecting group B streptococcal carriage.

The observed difference between carriers of group B streptococci identified by the selective broth medium (94%) compared with those detected by blood agar plates (51%) or by CNA agar plates (41%) is significant (greater than five times the standard error for the former and greater than seven times the standard error for the latter).

DISCUSSION

The prevalence of group B streptococci in the throat was similar in a large number of patients with and without upper respiratory symptoms in this study. Group B streptococci accounted for ca. 50% of all β-hemolytic streptococci isolated from each group. This suggests that the presence of these organisms in the throat is probably not related to clinical pharyngitis, although one cannot absolutely exclude the possibility that it could be. Further studies are required to distinguish simple carriage from infection by determining whether the presence of group B streptococci in the throat provokes an antibody response to various cellular antigens or such extracellular antigens as the nucleases of group B streptococci that are immunologically distinct from those of group A streptococci (P. Ferrieri, E. D. Gray, and L. W. Wannamaker, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, B45, p. 19).

Males and females had relatively similar pharyngeal carriage rates for group B streptococci. The throat should be considered another important reservoir for these bacteria, although a comparison with other studies indicates that the frequency of carriage at this site is less than that found in the genital tract or gastrointestinal tract (1, 5). There is no information available on the site sequence of acquisition and colonization with group B streptococci in adults.

The 12% pharyngeal carriage rate for group B streptococci found in this study is higher than that of previous studies (1, 3, 5, 6), suggesting that the group B streptococcus is a more frequent inhabitant of the throat than previously thought. The increased frequency of detection in this study is probably due to the use of multiple and more sensitive methods. If blood agar plates alone were used for cultures, the overall carriage rate would have been 6%, a rate similar to those reported previously.

This study supports other evidence that a selective broth enhances recovery of group B streptococci. If the broth were not utilized, 18 (37%) group B streptococcal carriers would have been undetected. On the other hand, if the selective broth medium alone were used, 94% of all the group B streptococcal carriers would have been detected. Mason et al. (9) have shown that the selective broth identified 97% of mothers and newborn infants carrying group B streptococci. Further, Baker et al. (2) ascertained, by using duplicate swabs, a group B

streptococcal vaginal carriage rate of 34% with the selective broth compared with 14% with a nonselective medium. The increased frequency of isolation of group B streptococci found by the use of a selective enrichment broth is probably related to aiding the growth of small numbers of organisms embedded in the swab. However, the clinical significance of this is not clear.

Ten percent of the group B streptococci isolated were bacitracin positive and would have been incorrectly identified as group A streptococci by this presumptive test. Wilkinson (12) found 6.2% of group B streptococci to be bacitracin positive. These findings suggest that other confirmatory tests (serological and/or biochemical) must be performed to avoid incorrect identification of group B streptococci.

With the increasing concern about the role of group B streptococci in neonatal pneumonia, sepsis, and meningitis, it is desirable that laboratories use isolation methods that optimize conditions for recovery of this organism. The use of a selective enrichment broth is a sensitive method for the recovery of group B streptococci from mucosal surfaces.

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