Optimal Site of Throat Swab for the Isolation of β -hemolytic Streptococci

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The optimal site for the isolation of β -hemolytic streptococci (BHS) from throat cultures was investigated in 164 healthy elementary school children. All throat cultures were streaked onto duplicate blood agar plates (BAP), one of which was taken from the tonsillar fossae and the other from the posterior pharynx. BHS were isolated in cultures from 56 (34.2%) of the children. BHS were more frequently recovered from the tonsillar fossae than from the posterior pharynx (54 vs. 47 ; both sites, 45 ; tonsillar fossae only, 9 ; posterior pharynx only, 2 ; $P < 0.0001$). There were significantly more numerous colonies in the tonsillar fossae than in the posterior pharynx ($p < 0.01$). To conclude, the tonsillar fossae are more optimal sites of throat cultures to isolate BHS than the posterior pharynx.

Key Words: Throat culture, Optimal site, β -hemolytic streptococci, Tonsillar fossae, Posterior pharynx

INTRODUCTION

The importance of streptococcal infections originates from the fact that they comprise a large segment of the treatable respiratory diseases and from their association with serious nonsuppurative sequelae, acute rheumatic fever and acute glomerulonephritis (Batlle and Glassgow, 1971 ; Wannamaker, 1972). Since no completely satisfactory clinical criteria are available for distinguishing patients with streptococcal respiratory infections with illnesses due to other agents, the routine use of throat cultures has been essential to the rational management of patients with respiratory infections (Batlle and Glassgow, 1971 ; Rosenstein et al., 1970). Many authors have given their efforts to enhance the isolation rate of β -hemolytic streptococci (BHS), but there are very few reports on the optimal sites of throat cultures to isolate BHS (Brien and Bass, 1985 ; Kaplan et al., 1979). The present

study was carried out to decide whether the tonsillar fossae or the posterior pharynx is more adequate for throat cultures by comparing the recovery rates of BHS cultured and the number of colonies of each site.

MATERIALS AND METHODS

Throat cultures were taken during March, 1993 in 164 healthy children in the 4th grade of a rural elementary school without signs or symptoms of upper respiratory tract infection. The throats were swabbed by rubbing a sterile cotton-tipped wooden applicator over the tonsillar fossae and the posterior pharynx in sequence. In this paper, the tonsillar fossae mean either tonsillar fossae or tonsillar surface. The swabs were then smeared directly onto sheep blood agar (BAP, Korea Media, Seoul, Korea) and streaked with a wire loop within 1 hour. After 18 to 24 hours incubation at 37 °C, the presence or absence of BHS on the BAP was observed. One of the surface colonies was subcultured and identified with Bacitracin disk (0.04 U, Becton Dickinson, USA) and a commercial latex agglutination kit (Seroiden Streptokit, Eiken, Tokyo, Japan). The number of colonies of BHS found on BAP were estimated and described as follows: 1+

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for less than 10 colonies; 2+ for 11 to 50 colonies; 3+ for 51 to 100 colonies; 4+ for more than 100 colonies, predominant or pure growth. This is a quantitative measurement of colonies that has been defined as 'the degree of positivity' (Breese et al., 1970 ; Stillerman and Bernstein, 1961).

RESULTS

Of the 164 throats cultured, 56 (34.2%) yielded BHS of which 50 were identified as group A. Only 45 of the 56 positives were recovered on both plates. Nine of 11 positives isolated from a single plate were recovered from the tonsillar fossae, two from the posterior pharynx (Table 1, χ^2 -test, $p < 0.0001$).

The correlation of frequency of the degree of positivity cultured from the tonsillar fossae and the posterior pharynx is shown in Table 2. In the thirty-two children who had more colonies of BHS in the tonsillar fossae, nine were no growth, twelve 1+, eight 2+, and three 3+ of the degree of positivity in the posterior pharynx. On the contrary, only nine children had more colonies of BHS in the posterior pharynx, of which two were no growth, three 1+, one 2+, and three 3+ of the degree of positivity in their tonsillar fossae respectively ($p < 0.01$).

Comparison of the number of colonies between the tonsillar fossae and the posterior pharynx shows that the relative incidence of cultures with a 1+ degree of positivity was markedly higher in the posterior pharynx ($p < 0.01$); a 2+ degree of positivity was approximately equal in the two groups; and a 3+ or 4+ was significantly higher in the tonsillar fossae (Fig. 1, $p < 0.0001$).

DISCUSSION

It is important that clinical investigators and practicing physicians continually review their guidelines for diagnosis of respiratory illness (Kellog, 1990 ; Wannamaker, 1972). Because the diagnosis of a streptococcal infection of the upper respiratory tract on clinical grounds is necessarily presumptive, a throat culture can provide a valuable laboratory clue (Wannamaker, 1965). Precision in the diagnosis of streptococcal infections of the upper respiratory tract has been improved by the more general availability and use of throat cultures (Breese et al., 1970 ; Wannamaker, 1972). It is known that throat cultures from patients with acute streptococcal infections are positive for BHS in over 95 percent of cases if adequately processed

Fig.1. Distribution of number of colonies of BHS(n=56) in tonsil and pharynx. No. of colonies : NG, no growth; 1+, <10 CFU; 2+, 10-50 CFU; 3+, 51-100 CFU; 4+, >100 CFU or pure growth.

(Wannamaker, 1965). Although a throat culture is a somewhat simple laboratory procedure to perform, a throat culture may be erroneous if it is not properly taken, processed, and interpreted (Kellog, 1990 ; Rosenstein et al., 1970 ; Wannamaker, 1965).

For epidemiologic purposes, a further check of accuracy of recovery rates is essential (Batlle and Glassgow, 1971 ; Rosenstein et al., 1970 ; Saslaw et al., 1959). We might consider variable factors affecting the recovery rates of BHS from the patients of acute pharyngitis, such as the carriage rate of the community (Wannamaker, 1972), patient's condition (exudate or pus on the pharynx) (Breese et al., 1970), technique of throat swab (illumination with a light, site for swab, vigorous rubbing or simple touch, contamination with oral mucosa or tongue, and the number of swabs), transport (or direct inoculation), media (enriched broth, or selective BAP) (Kellog, 1990 ; Wannamaker, 1965), incubation conditions (room air, CO₂, or anaerobic) and technical expertise (streaking, experienced eye, subculture, and identification) (Batlle and Glassgow, 1971 ; Rosenstein et al., 1970), etc. Of these variable factors, the author proceeded with this study focusing on the swab site from which the throat culture is obtained in the postulation that the distribution of BHS in throats between a carriage and the bona fide streptococcal infection is similar. Unfortunately, the result of swabs obtained simultaneously from the tonsillar fossae and the posterior pharynx is not compared in this study.

Some reports noticed that seasonal incidence

was highest in March and April (Stillerman and Bernstein, 1961), and age incidence was highest in 10 to 11 years old age (Kim et al., 1992) and both factors were considered in carrying out this study. The carriage rate of *S. pyogenes* in healthy children is known to be about 15-20%, and rates as high as 40% have been reported, (Bell and Smith, 1976 ; Kim et al., 1992 ; Wannamaker, 1972). From the above results, as carriage rate of *S. pyogenes* revealed as high as 31% (50/164) in the healthy school children of a rural area in Korea.

An adequate culture of the throat cannot be overemphasized. The tongue should be depressed and the throat adequately exposed and illuminated (Wannamaker, 1965). The swab should be rubbed well over each tonsillar area and/or the posterior pharynx. Care should be taken to avoid contaminating the swab by touching the tongue and oral mucosa (Brien and Bass, 1985 ; Wannamaker, 1965). The swab should be inoculated as soon as possible, at least within 2 hours of sampling. The swab should be immediately processed by rubbing and rotating on the one-sixth of a BAP (Wannamaker, 1965). The agar was not stabbed with a needle in this study (Belli et al, 1984). Inoculated plates are incubated at 37°C during overnight or for 48 hours (Hayden et al., 1984 ; Kellog, 1990). Some authors have reported that BHS were more frequently isolated in the anaerobic condition than in the aerobic or CO₂ incubation (Belli et al., 1984 ; Hayden et al., 1984 ; Schwartz et al., 1985). Because in mixed cultures of hemolytic staphylococci and streptococci, the more rapidly growing larger staphylococci may obscure the streptococci, careful observation and subculture technique are necessary. Other hemolytic gram-negative cocci or *Hemophilus hemolyticus* should be differentiated (Breese, 1978 ; Wannamaker,

1965).
The frequency of recovery of BHS from throat cultures has been known to depend on the number of swabs taken simultaneously because the single culture may induce a certain amount of error (Saslaw et al., 1959). But the cost and workload should be taken into account to expand the number of swabs. Saslaw et al. (1959) and Stillerman and Bernstein (1961) recommended that two swabs and two BAPs could be effectively used in throat culture to minimize the potential error of single swab. When the throat was swabbed twice at different sites of tonsillar fossae and posterior pharynx, the concordance rate between these two sites revealed as 93.3% (153/164) in this study. Because eleven strains of BHS (6.7%) would have been missed if only one swab had been used (Table 1), simultaneous swabbing on both sites would be necessary.

Brien and Bass (1985) compared the growth of *S. pyogenes* against the patients with streptococcal pharyngitis by culture site and found that tonsil, posterior pharyngeal wall, base of tongue, palate, and saliva etc. in order were adequate sites to isolate the organism. The result of this study agreed with their's in the point that growth of BHS was less on the posterior pharyngeal wall than on the tonsillar fossae. Becuase the number and the subjects were different as 12 selected patients with a heavy growth of *S. pyogenes* in Brien and Bass's study (1985) while 56 carriers of BHS in this study, the author could suggest that there are some differences of the degree of positivity between two studies. If it is difficult to swab the throat due to the lack of cooperation from a child, the base of tongue or saliva may become an alternative. The sensitivity and specificity of quantitative saliva cultures compared with throat cultures for *S. pyogenes* was well evaluated by Kaplan et

Table 1. Comparison of the isolation rate of β -hemolytic streptococci (BHS) between the tonsillar fossae and the posterior pharynx

Site	Tonsillar fossae		Total	(%)
	BHS-isolated	BHS-not isolated		
Posterior pharynx				
BHS-isolated	45	2	47	(29)
BHS-not isolated	9	108	117	(71)
Total	54	110	164	(100)
(%)	(33)	(67)	(100)	

Concordance rate, 93.3% (153/164)

χ^2 -test, p<0.0001

Table 2. The correlation of frequency of each number of colonies of β -hemolytic streptococci (BHS) between the tonsillar fossae and the posterior pharynx*

No. of colonies of BHS at posterior pharynx	No. of colonies of BHS at tonsillar fossae					Total
	NG	1+	2+	3+	4+	
NG			4	1	4	9
1+	1	3	5	4	3	16
2+		3	3	3	5	14
3+	1		1	1	3	6
4+				3	8	11
Total	2	6	13	12	23	56

* Number of colonies:see Fig. 1.
 χ^2 -test, $p<0.01$

al. (1979). They found that the throat culture was a more reliable means of identifying *S. pyogenes* in the upper respiratory tract than was the saliva culture. But the converse was true for non-group A BHS, that was, the saliva culture was a much better means for isolating these organisms.

Bacteriological examination is more of value clinically and epidemiologically when the results of cultures are quantitatively assessed (Breese et al., 1970; Breese, 1978; Stillerman and Bernstein, 1961). Small numbers of BHS are commonplace if very sensitive bacteriological methods are employed (Breese et al., 1970). Breese et al. (1970) found that significant clinical disease was usually associated with large numbers of BHS in the cultures. Our data of the proportion of the degree of positivity of BHS of healthy children are different from those of Breese et al. (1970) or Bell and Smith (1976), but the quantitative assessment of the number of streptococci isolated on culture has been regarded very importantly to distinguish the bona fide streptococcal infection from the carriage so far.

The adequacy of throat culture of isolation of BHS was investigated by taking throat cultures from 164 children. Fifty-four (33%) had BHS in the tonsillar fossae and 47 (29%) in the posterior pharynx respectively (Table 1, $p<0.0001$). The differences observed can be attributed to the number of colonies recovered (Table 2, $p<0.01$). Sixty-three percent (35/56) of the swabs of the tonsillar fossae were found to be in the 3+ to 4+ range of the degree of positivity while only 30% (17/56) of those of the posterior pharynx (Fig. 1, $p<0.0001$). From these results, we could conclude that the tonsillar fossae are more optimal sites than the

posterior pharynx for isolating BHS from throat cultures and simultaneous swabbing on both sites would be necessary to enhance the isolation rate of BHS.

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