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Influence of the Collection and Transport of Specimens on the Recovery of Bacteria from Peritonsillar Abscesses

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In 30 patients with peritonsillar abscesses, pus was obtained by aspiration and by taking a swab after incision; bacterial recovery was compared. Although processed in the laboratory within 2 h, swab specimens gave results comparable to syringe specimens in only 9 of 13 patients with beta-hemolytic streptococci and 7 of 25 patients with anaerobic bacteria. Both kinds of microorganisms were lost in some cases but appeared as additional flora in others. The poor results from the swab technique was ascribed to overgrowth of respiratory flora contaminating the sample after incision. In aspirated pus kept in the syringe, or transferred to anaerobic transporters, the microbial flora was unchanged for 24 to 48 h. Some anaerobes also survived on agar slants for 24 h, but specially designed anaerobic transporters are recommended.

Beta-hemolytic streptococci were recovered from peritonsillar abscesses in about 50% of the cases. Anaerobic bacteria were most frequently isolated in the remaining cases (A. Flodström and H. O. Hallander, Scand. J. Infect. Dis., in press; A. U. Hanson, Dissertation, Seruminstitutet, Copenhagen, 1950).

Anaerobic microorganisms, however, are also prevalent as indigenous flora in the throat. Thus there is a need for reevaluation of the methods used to collect specimens without contamination. It is also essential to choose transport systems that minimize the risk of exposure to oxygen and overgrowth of rapidly multiplying bacteria (1, 4, 11, 12, 14; K. Holmberg, Arch. Oral Biol., in press).

In a study on the microbiological etiology of peritonsillitis these problems were investigated in detail. The primary aim was to compare pus obtained by direct aspiration, by using a sterile needle and syringe, with pus from an incision, by swab sampling. Another purpose was to study the influence of commercially available transport systems on the survival of the original flora.

MATERIALS AND METHODS

Patients. The material for this part of the study comprised a total of 30 patients with unilateral peritonsillar abscesses. No patients had received antibiotic therapy within the last month.

Collection. Pus specimens were obtained by a syringe technique in which pus was aspirated with a hypodermic needle into an ordinary 2-ml syringe, or by a swab technique in which, after incision and debridement, pus was collected on a cotton-wool swab with or without activated charcoal.

Transport. Material collected by the syringe technique was transported by two methods: a syringe with a needle inserted in a rubber stopper (this method was used as reference); or an anaerobic transporter containing prereduced anaerobically sterilized (PRAS) salt solution containing 4 ml of resazurin (11 mg in 44 ml of distilled water) and 0.5 g of cysteine per 1,000 ml. The medium was enclosed in a diaphragm-stoppered glass tube allowing injection of pus. Oxygen was flushed out with a gas mixture of 97% CO_2 and 3% H_2 (8).

Material collected on swabs after an incision was transported by several methods. The first was an anaerobic transport medium with a column of PRAS semisolid medium. The medium was enclosed in nitrogen-filled glass ampoules that were opened immediately before use. The transport medium was made at the National Bacteriological Laboratory (SBL) and kindly made available by Carl-Erik Nord (1). In this system, a cotton-wool swab with activated charcoal (Nunc, Denmark) passed through a rubber stopper to exclude O_2 was used. The second method was by ordinary agar slants. This transport system was used with ordinary cotton-wool swabs (LIC, Sweden).

In a series of experiments, aspirated pus was transferred to swabs and kept in transport tubes for different times. In this study two more transport systems were tested. One was an SBL transport medium, a modified Stuart medium (6), enclosed in glass ampoules, as the swab technique transport method above. A cotton-wool swab with activated charcoal was included in this system. The second one was the Culturette system (Medi/Flex, Rockford, Ill., available through Biodisk, Stockholm), containing modified Stuart transport media. Transport vials of this type included a cotton-wool swab.

Culture procedures. All samples were cultured within 2 h after collection. The aerobic culture procedure included inoculation on blood, hematin (10% heated horse blood), phenol red-mannitol, and gen-

tian violet agar plates. The hematin and gentian violet agar plates were incubated in a thermostatically controlled incubator with 10% CO_2 at 37 C.

The anaerobic culture procedure included inoculation on freshly prepared PYG agar plates (8) and on freshly prepared blood agar plates (meat extract, 0.3 g; peptone, 1.0; NaCl, 0.6; NaH_2PO_4, 0.2 g; and horse blood, 10 ml; all per 100 ml). During the latter part of the investigation the blood agar was replaced by Brucella agar (BBL). Paper disks containing 30 μg of neomycin were used to select anaerobes. Anaerobic culture conditions were achieved with the Gas-Pak System (BBL) (3, 9).

All plates were incubated at 37 C for at least 48 h. For enrichment, PRAS PYG broth, flushed out with a mixture of 97% CO_2 and 3% H_2, was used. The mixture was buffered with 0.5 g of $NaHCO_3$ per 100 ml (8).

Identification. Anaerobic isolates were identified to the genus level by the Gram stain, colonial morphology, and gas chromatographic analysis of products of glucose fermentation according to the Virginia Polytechnic Institute (8). When organisms were identified by species, the API-system (13) (La Balme-Les-Grottes, France, available through Biodisk, Stockholm) was used.

The beta-hemolytic streptococci were serologically grouped by a capillary precipitation test performed with extracts prepared by the Fuller formamide method (5), and with specific group antisera from Burroughs Wellcome Co., London.

RESULTS

Recovery of bacteria from aspirated material versus swab material. In 30 patients, pus was obtained by aspiration as well as by the swab technique after incision. Samples were transported as shown in Table 1.

There was a striking difference in bacterial findings between the two techniques, although specimens were processed within 2 h. This is exemplified by the recovery of beta-hemolytic streptococci and anaerobic bacteria in two cases (Table 1). One case, using the syringe technique, was characterized by predominant growth of beta-hemolytic streptococci, group A. In this case no beta-hemolytic streptococci could be recovered from the swab material, but there was a heavy growth of commensals, including microaerophilic streptococci and bifidobacteria, anaerobes presumably appearing as contaminants in these circumstances. In a second case there was a pure culture of fusobacteria from aspirated pus. Those bacteria were also recovered from swabs, but not as single colonies as they were mixed with the respiratory flora. It was of special interest that beta-hemolytic streptococci, group G, appeared as additional flora from swab samples after transport on agar slants. This finding was interpreted as due to contamination from the mucous membranes, since the same bacteria were isolated in throat cultures.

The results from all 30 patients taken together showed that beta-hemolytic streptococci (Table 2) were isolated from pus collected and transported by the syringe technique in 13 cases; in 8 of these cases various anaerobes were also present. When collected on swabs, the beta-streptococci were not recovered in three of the cases recovered by the syringe technique. On the other hand, beta-hemolytic streptococci were recorded as additional flora twice after transport in SBL anaerobic medium, and once after transport on ordinary agar slants.

The same type of analysis was made for anaerobic bacteria (Table 3). Regardless of the collection and transport methods used, anaerobic microorganisms were isolated in a majority of cases. As a rule, more than one type of anaerobe was recorded from the same case. However,

TABLE 1. *Recovery of bacteria from aspirated pus and pus collected on swabs after incision in two cases of peritonsillar abscess*

Tonsil	Aspirate transported in:		Swab specimen transported in:	
	Syringe	Anaerobic transporter with PRAS salt solution	SBL anaerobic medium	Agar slant
Beta-hemolytic streptococci, group A	Aerobic: beta-hemolytic streptococci, group A	Aerobic: beta-hemolytic streptococci, group A	Aerobic: respiratory flora	Aerobic: respiratory flora
	Anaerobic: 0	Anaerobic: peptostreptococci	Anaerobic: microaerophilic streptococci, bifidobacteria	Anaerobic: microaerophilic streptococci, bifidobacteria
Beta-hemolytic streptococci, group G	Aerobic: 0	Aerobic 0	Aerobic: alpha-streptococci and others	Aerobic: beta-hemolytic streptococci, group G
Candida	Anaerobic: fusobacteria	Anaerobic: fusobacteria	Anaerobic: overgrowth of respiratory flora	Anaerobic: overgrowth of respiratory flora

there were marked qualitative differences between samples collected and transported by different techniques.

Material transferred from syringes to anaerobic transporters containing PRAS salt solution showed good correlation, though not identical results. Swab specimens gave results identical with those of syringe specimens in only seven cases when transported in SBL anaerobic medium and in six cases when transported on agar slants. In about half the number of cases anaerobes were lost, but they appeared just about as frequently as new flora as compared to aspirated specimens.

Almost invariably there was an admixture of aerobic respiratory flora as well, often resulting in complete overgrowth. No difference was noted between swab specimens transported in a special anaerobic transport medium and those transported on ordinary agar slants.

Pus collected and transported on swabs in SBL anaerobic medium was studied especially with regard to loss or addition of certain anaerobic species (Table 4). This study indicated no significant differences, however. Microbes lost in some cases appeared as contaminants in others. As a rule, microaerophilic streptococci were first isolated from anaerobic plates but were recorded as aerobes.

Influence of delay and transport technique on the recovery of bacteria from aspirated material. The results presented so far deal essentially with the aspirate versus the swab sampling method. To test the influence of delay on different transport modes and media, pus from one syringe was left on the bench at room temperature and plated at various intervals. Material from the same syringe was also transferred to swabs in different transport tubes, one swab and one tube to be used at each allotted time. In this part of the study the selection of transport systems was extended to cover two modifications of Stuart medium, SBL transport medium and Culturette.

In one example (Table 5) two biotypes of *Fusobacterium nucleatum*, no. 1 hemolytic and slightly more resistant than no. 2, were recovered anaerobically from aspirated pus along with sparse microaerophilic streptococci.

From earlier plated swabs *Fusobacterium* no. 1 dominated but later was replaced by biotype 2. This process occurred faster in the syringe than in the anaerobic transporter with PRAS salt solution, possibly due to pus being a good substrate. In SBL anaerobic transport medium both biotypes remained viable for 2 days, whereas type 1 was lost early in the modified Stuart media and on agar slants. In the latter

TABLE 2. *Recovery of beta-hemolytic streptococci from various transport systems compared to samples collected and transported by syringe technique in 30 patients with peritonsillar abscesses*

Determinants	Total recovery (no.)	Compared to syringe technique	
		Not recovered	Additionally recovered
Syringe technique	13		
Anaerobic transporter with PRAS salt solution	14	0	1
SBL anaerobic medium	12	3	2
Agar slant	11	3	1

TABLE 4. *Analysis of anaerobes lost and appearing as additional growth from swab specimens transported in SBL anaerobic medium and compared to syringe specimens*

Organism	Not recovered	Additional growth
Bacteroides	4	2
Fusobacteria	6	2
Veillonella	3	3
Bifidobacteria	1	1
Peptostreptococci	3	4
Peptococci	1	—

TABLE 3. *Recovery of anaerobic bacteria from various transport systems compared to samples collected and transported by syringe technique in 30 patients with peritonsillar abscesses*

Determinants	Total recovery (no.)	Compared to syringe technique		
		Same anaerobic recovery	One or more species not recovered	Additional recovery of one or more species
Syringe technique	25			
Anaerobic transporter with PRAS salt solution	26	22	4	5
SBL anaerobic medium	25	7	13	12
Agar slant	24	6	15	12

TABLE 5. *Recovery of anaerobes from aspirated pus related to delay in various transport systems*[a]

Transport system	Delay			
	1 h	18 h	42 h	66 h
Syringe specimen	Aerobic: ab alpha-streptococci sp *Neisseria* Anaerobic: ab fusobacteria 1 mo fusobacteria 2 sp microaerophilic 1	ab fusobacteria 1 mo fusobacteria 2 mo microaerophilic 1	sp fusobacteria 1 ab fusobacteria 2 ab microaerophilic 1	nr ab fusobacteria 2 ab microaerophilic 1
Anaerobic transporter with PRAS salt solution		ab fusobacteria 1 mo fusobacteria 2 nr	ab fusobacteria 1 mo fusobacteria 2 nr	nr ab fusobacteria 2 nr
SBL anaerobic transport medium		ab fusobacteria 1 mo fusobacteria 2 nr, sp microaerophilic 2(ag)	ab fusobacteria 1 mo fusobacteria 2 nr mo microaerophilic 2(ag)	nr ab fusobacteria 2 nr
SBL aerobic transport medium		ab fusobacteria 1 nr nr sp microaerophilic 2(ag)	nr nr nr ab microaerophilic 2(ag)	nr nr nr ab microaerophilic 2(ag)
Agar slant		nr nr nr ab microaerophilic 2(ag)	nr nr nr ab microaerophilic 2(ag)	nr sp fusobacteria 2 nr ab microaerophilic 2(ag)
Culturette		nr nr nr	nr nr nr	nr nr nr

[a] ab, abundant, >200 colonies; mo, moderate, 20 to 200 colonies; sp, sparse, 1 to 19 colonies; nr, not recovered; ag, additional growth.

medium microaerophilic streptococci soon became dominant.

In another example (Table 6), *F. nucleatum*, *Bacteroides pneumosintes*, and *Veillonella alcalescens* gave original yields for up to 2 days. Only on agar slant were the *Bacteroides* and *Veillonella* species lost after 24 h.

The same type of experiments on anaerobes performed with other patients produced similar results. In one case tested with beta-hemolytic streptococci, group A, in syringe pus, these bacteria were easily isolated after 48 h regardless of the transport system, although there was a moderate loss in Culturette vials.

DISCUSSION

To achieve reliable bacteriological results, the samples must be collected in such a way as to avoid contamination by normal flora and to prevent overgrowth during transport (1, 4, 7, 12, 14, 16; Holmberg, in press). For anaerobic conditions the samples must also be guaranteed an O_2-free atmosphere.

It is evident that samples for culture from peritonsillar abscesses collected after incision and debridement give insufficient or even misleading results, because of contamination by and overgrowth of the normal throat flora. Rapid transport, with samples processed within 2 h, also gave inadequate anaerobic results. Bedside plating might be an alterative in certain cases but cannot be considered routine. Instead, it must be recommended that specimens be aspirated in a syringe and the needle inserted in a rubber stopper, or pus should be injected into a special anaerobic transporter containing, for example, PRAS salt solution (8, 14). A certain degree of contamination may be inevitable, but this will probably be a minor problem with this technique if a rough quantitation is made. In the present study, anaerobic organisms were preserved in this manner in the original proportions for 24 to 48 h

TABLE 6. *Recovery of anaerobes from aspirated pus related to delay in various transport systems*[a]

Transport system	Delay			
	1 h	15 h	39 h	62 h
Syringe specimen	Aerobic: sp *Neisseria* Anaerobic: ab *Fusobacterium nucleatum* sp *Bacteroides pneumosintes* sp Veillonella alcalescens	sp *Neisseria* ab *F. nucleatum* ab *B. pneumosintes* mo *V. alcalescens*	mo *Neisseria* ab *F. nucleatum* ab *B. pneumosintes* mo *V. alcalescens*	mo *Neisseria* ab *F. nucleatum* nr nr
Anaerobic transporter with PRAS salt solution		sp *Neisseria* ab *F. nucleatum* mo *B. pneumosintes* mo *V. alcalescens*	sp *Neisseria* ab *F. nucleatum* mo *B. pneumosintes* mo *V. alcalescens*	nr sp *F. nucleatum* sp *B. pneumosintes* sp *V. alcalescens* sp microaerophilic streptococci (ag)
SBL anaerobic transport medium		nr ab *F. nucleatum* ab *B. pneumosintes* sp *V. alcalescens*	nr ab *F. nucleatum* ab *B. pneumosintes* nr mo microaerophilic streptococci (ag)	nr ab *F. nucleatum* nr sp *V. alcalescens*
SBL aerobic transport medium		ab *F. nucleatum* mo *B. pneumosintes* mo *V. alcalescens*	ab *F. nucleatum* mo *B. pneumosintes* mo *V. alcalescens*	ab *F. nucleatum* nr sp *V. alcalescens*
Agar slant		ab *F. nucleatum* sp *B. pneumosintes* sp *V. alcalescens*	sp *F. nucleatum* nr nr	sp *F. nucleatum* nr nr

[a] ab, abundant, >200 colonies; mo, moderate, 20 to 200 colonies; sp, sparse, 1 to 19 colonies; nr, not recovered; ag, additional growth.

before changes, frequently involving overgrowth of fusobacteria were noted. Beta-hemolytic streptococci were less sensitive in this respect and also were usually identified in contaminated samples.

Some anaerobes are rather tolerant towards oxygen (2, 10, 15). In this study, for example, 48-h survival on agar slants was demonstrated for some species of *Fusobacterium* as well as *Bacteroides*. It is interesting to note that anaerobic organisms also appeared as contaminants after transport on agar with free exposure to air. Better recovery of anaerobes is possible, however, if specially designed anaerobic transport systems are used.

Cotton swabs have been reported to contain substances with antibacterial activity. This adverse effect has been reduced by adding albumin or activated charcoal (2, 6). In this study swabs included in commercially available transport systems were used. The system was studied as such and no separate conclusion could be made for the swabs.

LITERATURE CITED

1. Bäck, E., and C.-E. Nord. 1973. Anaeroba infektioner. Läkartidningen 72:1199–1205.
2. Collee, J. G., B. Watt, R. Brown, and Sandra Johnstone. 1974. The recovery of anaerobic bacteria from swabs. J. Hyg. Cambridge 72:339–347.
3. Collee, J. G., B. Watt, E. B. Fowler, and R. Brown. 1972. An evaluation of the Gaspak system in the culture of anaerobic bacteria. J. Appl. Bacteriol. 35:71–82.
4. Finegold, S. M., and J. E. Rosenblatt. 1973. Practical aspects of anaerobic sepsis. Medicine 52:311–322.
5. Fuller, A. T. 1938. The formamide method for extraction of polysaccharides from haemolytic streptococci. Br. J. Exp. Pathol. 19:130.
6. Gästrin, B., L. O. Kallings, and A. Marcetic. 1968. The survival time for different bacteria in various transport media. Acta Pathol. Microbiol. Scand. 74:371–380.
7. Gorback, S. L., and J. G. Bartlett. 1974. Anaerobic infections. N. Engl. J. Med. 290:1177–1184, 1237–1245, 1289–1294.
8. Holdeman, L. V., and W. E. G. Moore. 1973. Anaerobic laboratory manual. The Virginia Polytechnic Institute and State University Anaerobe Laboratory. Blacksburg, Va.
9. Killgore, G. E., S. E. Starr, V. E. Del Bene, P. N. Whaley, and V. R. Dowell, Jr. 1973. Comparison of three anaerobic systems for the isolation of anaerobic bacteria from clinical specimens. Am. J. Clin. Pathol. 59:552–559.
10. Loescke, W. J. 1969. Oxygen sensitivity of various anaerobic bacteria. Appl. Microbiol. 18:723–727.
11. Martin, N. J. 1974. Isolation and identification of anaerobic bacteria in clinical laboratory. Mayo Clin. Proc. 49:300–308.

12. Rosenblatt, J. E., A. Fallon, and S. M. Finegold. 1973. Comparison of methods for isolation of anaerobic bacteria from clinical specimens. Appl. Microbiol. 25:77–85.
13. Starr, S. E., F. S. Thompson, V. R. Dowell, Jr., and A. Balows. 1973. Micromethod system for identification of anaerobic bacteria. Appl. Microbiol. 25:713–717.
14. Sutter, V. L., H. R. Attebery, J. E. Rosenblatt, K. S. Brickwell, and S. M. Finegold. 1972. Anaerobic bacteriology manual. University of California, Los Angeles.
15. Tally, P. F., P. R. Stewart, V. L. Sutter, and J. E. Rosenblatt. 1975. Oxygen tolerance of fresh clinical anaerobic bacteria. J. Clin. Microbiol. 1:161–164.
16. Yrios, J. W., E. Balish, A. Helstad, C. Field, and S. Inhorn. 1975. Survival of anaerobic and aerobic bacteria on cotton swabs in three transport systems. J. Clin. Microbiol. 1:196–200.