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Survival of Anaerobic and Aerobic Bacteria on Cotton Swabs in Three Transport Systems

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The capacity of aerobic and anaerobic bacteria to survive on cotton swabs placed into a dry gassed-out CO_2-filled tube (DGT), a dry sterile aerobic tube (DAT), and a tube containing a modified Stuarts' transport medium (MST), was assessed. *Pseudomonas aeruginosa* increased in numbers by 2 and 3 logs when stored in MST and DAT, respectively. The viability of *P. aeruginosa*, although retarded when compared to MST and DAT, was not adversely affected by the CO_2 environment in the DGT. The MST maintained relatively constant numbers of *Streptococcus pyogenes* during the 48-h storage period. The DAT and the DGT were unable to maintain the viability of *S. pyogenes*. *Staphylococcus aureus*, when stored in a DGT, DAT, or MST, was maintained in relatively constant numbers throughout the entire storage period. Of the four anaerobic bacteria evaluated (*Bacteroides fragilis* ssp. *thetaiotaomicron*, *Bacteroides melaninogenicus* ssp. *asaccharolyticus*, *Fusobacterium nucleatum*, and *Peptostreptococcus anaerobius*), only *B. fragilis* ssp. *thetaiotamicron* survived the 48-h storage period in the DGT. Under these test conditions the DGT did not adequately maintain the viability of the majority of anaerobic bacteria tested (when held on cotton swabs). However, the MST did maintain the viability of all species tested for at least the first 2 h of storage.

Transportation of anaerobic specimens to an anaerobic bacteriology laboratory is an extremely important link for the successful culture of pathogenic anaerobic bacteria. It has been generally accepted that injecting an aspirated fluid, from an infected site, immediately into a gassed-out (oxygen-free) tube or vial is the best method for the transport of anaerobic specimens (5, 8-10). Anaerobic specimens are often transported to the anaerobic bacteriology laboratory on cotton swabs. The survival of aerobic and anaerobic bacteria, on cotton swabs, has been studied by several workers using a variety of transport media (1, 3, 4, 6, 11). This study was undertaken to compare the gassed-out tube method of transport to that of a transport medium. We used colony-forming units (CFU) as a quantitative index of survival. (J. W. Yrios submitted a thesis in partial fulfillment of the requirements for the degree of Master of Science in medical microbiology at the University of Wisconsin, Madison, 1973. A portion of this paper was presented at the annual meeting of the American Society of Microbiology held in Chicago, Ill., 16 May 1974.)

MATERIALS AND METHODS

Bacteria. The following bacteria were chosen for this study since they represent a flora which could possibly be isolated from a wound or abscess. Care was taken to include an aerobic bacterium (*Pseudomonas aeruginosa*), two facultative bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*), and four anaerobic bacteria (*Bacteroides fragilis* ssp. *thetaiotaomicron*, *Bacteroides melaninogenicus* ssp. *asaccharolyticus*, *Fusobacterium nucleatum*, and *Peptostreptococcus anaerobius*). All bacteria were obtained from stock cultures maintained at the Wisconsin State Laboratory of Hygiene, Madison, Wisc.

Gassed-out tubes and swabs. Gassed-out tubes were culture tubes (18 by 142 mm) (Bellco Glass Inc., Vineland, N.J.) fitted with a number 1 black butyl rubber stopper. The air inside the tubes was replaced with oxygen-free CO_2, using the set-up recommended by the Virginia Polytechnic Institute Anaerobic Laboratory, Blacksburg, Va. The CO_2 gas was passed over hot copper turnings to remove any possible traces of oxygen. The tubes were gassed for approximately 1 min, tightly stoppered, secured in a press, and autoclaved (121 C, 30 min). The anaerobic environment of the tubes was checked by using a prereduced liquid medium with resazurin (Eh = -42 mV) in several tubes of each new batch of gassed-out tubes. The swabs (plain cotton-tipped with a wooden applicator) were placed into similar (18 by 142 mm) tubes, gassed-out, and sterilized. The dry sterile aerobic tubes (DAT) were also culture tubes (18 by 142 mm) containing air.

Transport medium. The transport medium used in this study is the same as that used by the

Wisconsin State Laboratory of Hygiene for mailed clinical specimens. It is basically a modified Stuarts' transport medium (MST) (7), containing inorganic phosphate buffers, NaCl, sodium thiogycollate, methylene blue indicator, and 1% agar. The medium was boiled and dispensed in 3-ml amounts into glass tubes (13 by 100 mm). The tubes, each with a cotton plug, were autoclaved (121 C, 15 min) and stored at room temperature for no more than 24 h before use.

Culture, dilution, and plating media. All stock cultures of the aerobic and the facultative bacteria were cultured in 5-ml quantities of brain heart infusion broth (BHI; Difco), diluted 10-fold in 4.5 ml of BHI, and assayed by plating on 7.5% sheep blood agar plates (veal infusion agar; BBL). The broth and plates were used within 5 days after preparation. The plates for the assay of *S. pyogenes* were incubated for 24 h in a 37 C — CO_2 incubator, while the assay plates for *S. aureus* and *P. aeruginosa* were incubated for 24 h in a 37 C aerobic incubator.

All stock cultures of the anaerobes were cultured in 5-ml quantities of prereduced peptone-yeast-glucose broth (PYG) supplemented with a hemin-menadione working solution (8), diluted in 4.5 ml of PYG, and assayed on fresh (3 h) 7.5% sheep blood agar plates (BHI agar with yeast extract [Difco], 1% hemin-menadione working solution, and cysteine hydrochloride). The latter plates were incubated at 37 C in a GasPak anaerobic jar (BBL). Anaerobic conditions inside the jars were attained using disposable gassing envelopes (BBL) in the presence of a fresh palladium catalyst.

Sample dilution and plating media. The quantitative technique of Bartlett and Hughes (2) was modified and used in this study. A pure 24-h broth culture of each organism was adjusted to a turbidity corresponding to a MacFarland no. 1 standard. The suspension was mixed thoroughly on a Vortex mixer (Vortex-Genie, Fischer Scientific, Pittsburgh, Pa). Ten 0.05-ml drops were dispensed on the inside of a sterile plastic petri dish (16 by 125 mm) and 1 drop was added to a tube containing 5 ml of broth (control). Within 2 min, 10 sterile cotton swabs from a dry gassed-out CO_2-filled tube (DGT) were used to absorb the 10 drops. The swabs were immediately placed into the appropriate transport container (three gassed-out tubes, three dry aerobic tubes, and three tubes of transport medium) and the time-zero swab was placed into 5 ml of broth for immediate assay by the plate count dilution method. A set of three swabs (one swab from each transport method) was assayed at 2 h, a second set at 24 h, and a third set at 48 h.

The swabs were mixed (Vortex mixer; setting no. 4) for 20 s in the stoppered tube containing 5 ml of the appropriate broth (BHI or PYG), "rung-out" on the inside of the tube, and discarded. Data from this study indicated that approximately 78% of the bacteria could be recovered from the swab by mixing with a Vortex mixer. Tenfold dilutions were carried out through a series of five more tubes containing 4.5 ml of either BHI or PYG broth. Two assay plates were inoculated with 0.1 ml of each dilution. The inoculum was spread out upon the agar surface using a Belgian stainless steel wire (size 0.031; Herters Inc., Waseca, Minn.) bent to give maximum spreading efficiency. All aerobic plates were incubated for 24 h while anaerobic plates were incubated for 48 h (except *B. melaninogenicus* ssp. *asaccharolyticus*; it had to be incubated for 5 days). After the appropriate incubation period the plates which contained 30 to 300 colonies were counted, averaged, and expressed as CFU per milliliter of the original adjusted inoculum.

RESULTS

P. aeruginosa. The aerobe, *P. aeruginosa*, when held on a cotton swab for 48 h in an aerobic environment [DAT], increased in numbers by approximately 3 logs (Table 1). In the transport medium (a reduced environment), the organism still increased in numbers by 2 logs in 48 h of storage. The least amount of growth was observed when *P. aeruginosa* was held in the CO_2 atmosphere of the gassed-out tube (1 log increase in 48 h).

S. pyogenes. The MST was originally used for the transport of throat swabs in the streptococcus surviellance program for the eradication of rheumatic heart disease (7). The MST maintained the streptococcus quite well over a 48-h storage period (Table 2). In the DGT, the organism decreased in numbers by almost 4 logs at 48 h. In the DAT, *S. pyogenes* dropped from 10^7 organisms per ml at time zero to less than 10^3 at 48 h, indicating a possible negative effect that desiccation might have when the bacteria are held on a cotton swab. We could not detect less than 1.0×10^3 bacteria per ml in our assay system. One colony on the assay plate at the lowest dilution represented at least 1.0×10^3 CFU per ml in the original inoculum.

S. aureus. There seemed to be little difference in the recovery of *S. aureus* from a cotton swab stored in either of the three methods of transport (Table 2). At 48 h of storage in a DAT, there was approximately a 1 log increase in recovered organisms; the MST yielded slightly less than a 1 log increase and the DGT produced the slightest increase (0.5 log).

B. fragilis ssp. thetaiotaomicron. The number of *B. fragilis* ssp. *thetaiotaomicron* which were recovered from a cotton swab stored in a DGT remained relatively constant throughout the 48-h storage period (Table 3). A 1 log decrease, within 24 h, was observed while the anaerobe was stored in MST. No viable organisms were recovered at 24 h of storage in a DAT and at 48 h in the MST.

B. melaninogenicus ssp. asaccharolyticus. After storage for 2 h, the number of recoverable *B. melaninogenicus* ssp. *asaccharolyticus* remained virtually unchanged in the MST and decreased by 2 logs and 3 logs, in the DGT and DAT, respectively (Table 3). At 24 h, no bacte-

TABLE 1. *The number of CFU of Pseudomonas aeruginosa recovered at various times from plain cotton swabs that were stored in a DGT, DAT, or a tube of MST*

Organism	Time (h)	Transport method		
		DGT	DAT	MST
Pseudomonas aeruginosa	0	1.6×10^8 [a]	4.8×10^8	1.6×10^8
	2	3.4×10^8	4.6×10^8	1.5×10^8
	24	6.8×10^8	1.0×10^{11}	2.0×10^{10}
	48	1.7×10^9	3.0×10^{11}	3.3×10^{10}

[a] Number of surviving bacteria (CFU) at designated time.

TABLE 2. *The number of CFU of Streptococcus pyogenes and Staphylococcus aureus recovered at various times from plain cotton swabs that were stored in a DGT, DAT, or a tube of MST*

Organism	Time (h)	Transport method		
		DGT	DAT	MST
Streptococcus pyogenes	0	8.6×10^7 [a]	1.6×10^8	8.6×10^7
	2	2.7×10^8	1.6×10^8	2.0×10^8
	24	1.2×10^7	1.7×10^6	2.5×10^9
	48	5.4×10^4	$< 1.0 \times 10^3$ [b]	2.0×10^9
Staphylococcus aureus	0	6.1×10^7	3.7×10^7	6.1×10^7
	2	7.5×10^7	5.8×10^7	1.1×10^8
	24	8.8×10^7	2.6×10^8	2.7×10^8
	48	1.6×10^8	3.9×10^8	3.2×10^8

[a] Number of surviving bacteria at designated time (CFU).
[b] The assay system used could not detect less than 1.0×10^3 CFU per ml.

ria were recovered from the DAT, the DGT decreased by another log (a 3 log decrease overall), and the viability in the MST had dropped by 3 logs. At 48 h, no viable bacteria were demonstrated in any of the methods of transport used in this study.

F. nucleatum. At 2 h, in a DAT, no CFU of *F. nucleatum* could be recovered from the cotton swab, a decrease of 2 logs was observed in the MST, and a 4 log decrease resulted from storage in the DGT. No organisms were recovered from cotton swabs stored for either 24 or 48 h in any of the methods of transport (Table 3).

P. anaerobius. At 2 h of storage, MST yielded about the same number of viable *P. anaerobius* as it did at time zero; however the DAT and the DGT showed at least a 1 log decrease in the number of viable bacteria (Table 3). At 24 h, the number of bacteria recovered from the MST decreased slightly, while the viable counts in the DAT and DGT decreased by 4 logs. At 48 h, no organisms were recovered from either the DGT or the DAT, but 10^3 viable counts were preserved in MST.

DISCUSSION

When compared to MST and DAT, the anaerobic atmosphere in the DGT appeared to suppress the growth of *P. aeruginosa* during the 48 h of storage. This growth suppression would be a desired characteristic of an effective transport medium. To determine if the suppression of growth is not the exception, but the rule for aerobes, the survival of several more aerobic bacteria would have to be studied under similar conditions of storage in the DGT. The MST allowed more growth of *P. aeruginosa* then did the DGT. However, fewer viable bacteria were present in MST than in DAT. Therefore, MST, which contained a reduced environment, was also able to suppress the growth of *P. aeruginosa*. The DAT was included in this study as a control, to check the inherent growth which takes place by each test organism contained in the 0.05 ml of nutrient broth on the cotton swabs. The containment of the test bacteria (on a swab) in a small amount of nutrient broth (as opposed to a buffered saline solution) probably more closely simulates the environment of a wound or abscess, which would contain nutrients such as effete cells, serum, or mucous.

The facultative bacteria studied, *S. pyogenes* and *S. aureus*, yielded quite different results on the three methods of transport. The MST showed a 1.5 log increase in viable *S. pyogenes* at 48 h of storage, probably due to the added nutrients supplied by the surrounding broth. The decrease in recoverable *S. pyogenes* from

TABLE 3. *The number of CFU of anaerobic bacteria recovered at various times from plain cotton swabs that were stored in a DGT, DAT, or a tube of MST.*

Organism	Time (h)	Transport methods		
		DGT	DAT	MST
Bacteroides fragilis ssp. *thetaiotaomicron*	0	5.1×10^6 [a]	5.1×10^6	5.1×10^6
	2	3.4×10^6	1.0×10^7	3.5×10^6
	24	3.1×10^6	$<1.0 \times 10^3$ [b]	3.1×10^4
	48	1.0×10^6	$<1.0 \times 10^3$	$<1.0 \times 10^3$
Bacteroides melaninogenicus ssp. *asaccharolyticus*	0	3.3×10^6	3.3×10^6	3.3×10^6
	2	4.6×10^4	5.0×10^3	2.4×10^6
	24	2.0×10^3	$<1.0 \times 10^3$	3.0×10^3
	48	$<1.0 \times 10^3$	$<1.0 \times 10^3$	$<1.0 \times 10^3$
Fusobacterium nucleatum	0	1.2×10^7	1.2×10^7	1.2×10^7
	2	1.0×10^3	$<1.0 \times 10^3$	3.2×10^5
	24	$<1.0 \times 10^3$	$<1.0 \times 10^3$	$<1.0 \times 10^3$
	48	$<1.0 \times 10^3$	$<1.0 \times 10^3$	$<1.0 \times 10^3$
Peptostreptococcus anaerobius	0	3.0×10^7	3.0×10^7	3.0×10^7
	2	4.1×10^5	1.0×10^7	1.1×10^7
	24	1.0×10^3	1.0×10^3	9.0×10^6
	48	$<1.0 \times 10^3$	$<1.0 \times 10^3$	1.0×10^3

[a] Number of surviving bacteria at designated time (CFU).
[b] The assay system used could not detect less than 1.0×10^3 CFU per ml.

the dry tubes (DGT and DAT) at 24 and 48 h indicates a possible negative effect of desiccation on some strains of *S. pyogenes*. The CO_2 atmosphere of the DGT appeared to counteract this latter negative effect (desiccation) because when compared to the DAT, consistently better viability of *S. pyogenes* was observed at 24 and 48 h in the DGT tubes. The need for a 5 to 10% atmosphere of CO_2 for the cultivation of some streptococci is a possible explanation for this increased recovery of *S. pyogenes* from the DGT. The number of viable *S. aureus* which could be recovered from either of the three methods of transport remained relatively stable throughout the 48-h period. The 1 log increase of *S. aureus* is probably again attributable to the nutrient broth in the inoculum.

Of the anaerobic bacteria studied, only *B. fragilis* ssp. *thetaiotaomicron* survived the 48-h storage in the DGT. At 2 h of storage, the MST yielded approximately 2 logs more viable *B. melaninogenicus* ssp. *asaccharolyticus*, *F. nucleatum*, and *P. anaerobius* than did the DGT. (MST and DGT yielded identical numbers of viable *B. fragilis* ssp. *thetaiotaomicron* at 2 h.) This would indicate that a transport medium is indeed very useful for maintaining the viability of some anaerobic bacteria on a cotton swab if plating is done immediately upon receipt by the laboratory.

The transport medium (MST) lost its anaerobic conditions, as measured with methylene blue, within 1 h after insertion of the cotton swab. Improved recovery of anaerobes could possibly be accomplished by decreasing the concentration of agar, by using a deeper column of transport medium, or by using a prereduced transport medium contained inside a glass tube fitted with an air-tight screw cap. All of these previous variations have been mentioned in the literature (10). However, it would require further study to evaluate the effects of these modifications upon the viability of anaerobic bacteria.

Cotton swabs were used in this study mainly for two reasons; (i) they are commonly used to collect and ship a specimen which might contain possible pathogenic anaerobic bacteria to the Wisconsin State Laboratory of Hygiene, and (ii) cotton has been shown to be more absorbant than calcium alginate, thus minimizing desiccation and oxidation which can be lethal to certain bacterial species. Our ability to recover (Vortex mixing) viable bacteria from the fibers of the cotton swab appeared to be quite adequate (78%).

The gassed-out tube used in this study was allowed to remain relatively dry because of possible technical problems in absorbing the inoculum onto already wet swabs. Holdeman and Moore (8) suggest the use of several drops of a salts solution to add moisture to the transport tube. The anaerobic conditions in the gassed-out tubes, as measured in our laboratory with

resazurin, was lost approximately 1 h after the introduction of the cotton swabs.

In conclusion, the aerobic and facultative bacteria studied (*P. aeruginosa* and *S. aureus*) were not adversely affected by any of the methods of transport. *S. pyogenes* did lose viability when stored in the DAT and DGT during the 48-h storage period. The viability of the majority of the anaerobic bacteria studied, when held on cotton swabs, was not adequately maintained in the dry gassed-out tube. The MST retained the viability of all the anaerobic bacteria tested for at least 2 h of storage. The use of MST for the transport of anaerobic bacteria, if modified in appropriate ways, could possibly increase its value as an anaerobic transport method.

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