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Effects of Storage in an Anaerobic Transport System on Bacteria in Known Polymicrobial Mixtures and in Clinical Specimens

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An anaerobic transport system (ATS) which provides for catalytic removal of oxygen was evaluated by using in vitro-prepared polymicrobial mixtures of log-phase bacteria and clinical specimens. Inoculated swabs were stored at room temperature in (i) aerobic, (ii) anaerobic glove box, and (iii) ATS environments, and bacteria were quantitated after 2, 24, 48, and 72 h. Bacteria in a three-part mixture of *Bacteroides fragilis, Peptostreptococcus anaerobius,* and *Escherichia coli* and in a five-part mixture of *B. fragilis, P. anaerobius, Fusobacterium nucleatum, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa* survived 72 h of storage in the ATS and anaerobic glove box environments, but the anaerobic species were inactivated in the aerobic storage except for *B. fragilis* in pure culture or in the three-part mixture. Changes in relative proportions among the species in a mixture were least in the ATS and anaerobic glove box environments and greatest during the aerobic storage, particularly in the five-part mixture. Bacteria present in pure or mixed culture in clinical specimens generally survived 72 h of storage in the ATS. These data indicate that changes in relative proportions occur with prolonged storage even under anaerobic conditions, but that the ATS would be most effective for preserving anaerobic bacteria and preventing drastic concentration changes and overgrowth of facultative and aerobic bacteria.

Protection of anaerobic bacteria from the deleterious effects of oxygen exposure and drying during transport of clinical specimens to the laboratory is an essential component of adequate processing for cultivation of anaerobes. Various transport media, gassed-out tubes and vials, GasPak jars (Baltimore Biological Laboratory, Cockeysville, Md. [BBL]), and syringe techniques have been utilized to overcome these problems (1, 3, 4, 7, 10, 15). Although material obtained by aspiration is preferable (2, 5, 7), nevertheless many clinical specimens are still transported on a swab. A recently described (14) anaerobic transport system, Anaerobic Specimen Collector (Becton, Dickinson & Co., Rutherford, N.J.), possesses certain theoretical advantages compared with gassed-out tubes containing swabs in which the entire stopper of the tube is removed during insertion of the specimen. This new anaerobic transport system (ATS) limits the amount of air entering with the clinical specimen such that the oxygen content within the tube is less than 2% immediately after insertion of the specimen. The ATS contains 10% hydrogen in an anaerobic gas mixture (balance, 80% nitrogen and 10% carbon dioxide) which in the presence of a palladium catalyst packet reduces the oxygen introduced into the system, reestablishing anaerobic conditions. A resazurin indicator disk is also included. The ATS can be used for transport of tissue samples or aspirated liquid specimens in addition to swabs. Initial tests of survival of pure cultures of anaerobic, facultative, and aerobic bacteria in the ATS for periods up to 48 h were successful (14).

The present study was designed to further evaluate the clinical usefulness of the ATS. Experiments were performed on mixed populations of bacteria prepared in vitro to simulate clinical material and on actual clinical specimens. Tests on prepared mixtures determined the survival of strains of anaerobic, aerobic, and facultative bacteria after exposures for periods up to 72 h to three different environments, aerobic (AER), anaerobic glove box (AGB), and the ATS. Because the majority of anaerobic infections are polymicrobial in nature, the experiments with bacterial mixtures were quantitative to evaluate the efficacy of the ATS in maintaining not only the same total bacterial count but also the viability of each of the species and, importantly,

the proportionality among species as originally present in the mixture. Survival of organisms in fresh clinical material was determined after storage in the ATS for periods up to 72 h. Results of testing of prepared mixtures and actual clinical specimens were compared.

MATERIALS AND METHODS

Bacterial culture. Two different polymicrobial mixtures were tested in an attempt to cover a range of oxygen tolerances with species of bacteria commonly isolated from clinical specimens. The first mixture contained *Bacteroides fragilis* NCDC 10946, *Peptostreptococcus anaerobius* VPI 5041, and *Escherichia coli* ATCC 25922, providing two anaerobic species (one aerotolerant and the other oxygen sensitive) and a facultative species. The second mixture consisted of *B. fragilis, P. anaerobius, Fusobacterium nucleatum* NCDC 10206, *Staphylococcus epidermidis* ATCC 12228, and *Pseudomonas aeruginosa* ATCC 14207. This mixture provided an additional oxygen-sensitive anaerobe, another facultative organism, and an aerobic species. Each organism was also tested in pure culture as a control. Certain of the anaerobic strains used in the three-part mixture were purposefully repeated in the five-part mixture to determine whether viability was dependent on the types of bacteria with which these organisms were concomitantly exposed. The anaerobic strains were human clinical isolates. All strains were maintained as frozen samples prepared from cultures of lyophilized stocks. Anaerobic organisms were cultivated within an anaerobic glove chamber (Coy Manufacturing Co., Ann Arbor, Mich.) containing 85% nitrogen, 10% hydrogen, and 5% carbon dioxide. Aerobic and facultative organisms were incubated aerobically but were placed in the anaerobic glove box for preparation of mixed inocula.

Preparation of inocula and exposure of swabs. Log-phase cultures of the above species were combined within the anaerobic glove box to give the three- or five-part mixtures. Individual broth cultures were previously diluted in broth as required so that a swab would contain approximately 10^5 to 10^6 bacteria of each species when exposed in polymicrobial mixtures or as a pure culture. Adjustment of the initial bacterial count to this midrange allowed for determination of increases or decreases in counts with time. From the adjusted bacterial suspension (mixed or pure), 0.1-ml samples were transferred in separate droplets to the surface of a petri dish. Dishes containing these inocula were passed outside of the anaerobic glove box, and to simulate actual clinical routine, the swabs were charged under aerobic conditions. Each swab was held in an individual droplet for 3 s until liquid was absorbed. The charged swabs were then placed in AER, AGB, and ATS environments. Two other swabs were immediately (zero time) quantitated to determine the initial count of each of the species on the swabs. The ATS and AER tubes were held in room air and ambient temperature in the dark. The AGB tubes were passed into the anaerobic glove box and also placed in the dark at room temperature. At each time interval, 2, 24, 48, and 72 h, one tube from each environment was passed into the anaerobic glove box, and the swabs were quantitated to determine the number of viable organisms.

Configuration of swabs for environmental exposure. Swabs from the ATS were utilized throughout these experiments so that the swab material would be consistent. Also the tube configuration of a swab within a small (inner) tube which was contained inside a larger (outer) tube was maintained for all environments to avoid any possible effect resulting from physical differences during the exposures. To prepare the ATS tubes to receive swabs intended for the AGB and AER exposures, the gray stopper was removed from the ATS and the inner tube containing the swab was separated from the stopper. The catalyst packet was removed from the large tube, but the resazurin indicator disk was left in place. The inner tube containing the swab was replaced inside the large tube, which then was covered with a sterile metal cap (Morton closure with pressure fingers), allowing interchange of ambient gas with the interior of the tube (Fig. 1a). The moisture content inside these AER and AGB tubes was maintained to match the moisture content within the ATS. For the ATS environment the Becton, Dickinson & Co. Anaerobic Specimen Collector was used exactly per instructions (Fig. 1b).

Quantitation of bacteria. Quantitation of bacteria from swabs (or in broth suspension) was performed within the anaerobic glove box. The inoculum was removed from each swab by mixing in 1.9 ml of freshly prepared Trypticase soy broth for 15 s on a Vortex mixer. Tenfold dilutions of the bacterial suspension were prepared, and duplicate 0.1-ml samples were spread on the surface of appropriate plate media. Plates were incubated at 37°C, aerobically for aerobic and facultative bacteria and anaerobically in the glove box for anaerobic bacteria. Plates containing 30 to 300 colonies were counted, and counts from duplicate plates were averaged.

Culture media. Anaerobic broth cultures for inoculation of swabs were grown in fluid thioglycolate medium without indicator-135C (BBL) supplemented with 0.2% yeast extract, 0.5 μg of menadione per ml, 5 μg of hemin per ml, and 10% rabbit serum. Aerobic broth cultures were grown in Trypticase soy broth (BBL). Sterile glass beads were included in cultures of *P. anaerobius* and *P. aeruginosa,* and these cultures were mixed on a Vortex mixer to disrupt long chains or aggregates for more accurate quantitation.

To determine the viable count of each species in polymicrobial mixtures, log dilutions were plated on a variety of media. Quantitation of *E. coli, P. aeruginosa,* and *S. epidermidis* was performed by using aerobic sheep blood agar plates (Trypticase soy agar [BBL] plus 5% defibrinated sheep blood). *B. fragilis* and *F. nucleatum* were quantitated on anaerobic blood plates containing vancomycin (brucella agar [BBL] supplemented with 0.5 μg of menadione per ml, 5 μg of hemin per ml, 5% defibrinated sheep blood, and 7.5 μg of vancomycin per ml). *P. anaerobius* was quantitated on anaerobic phenylethyl alcohol agar (BBL) supplemented with 0.5% yeast extract, menadione and hemin as above, and 5% defibrinated sheep blood. Pure cultures were quantitated by using aerobic or anaerobic sheep blood agar plates as required. After preparation, all anaerobic media were immediately

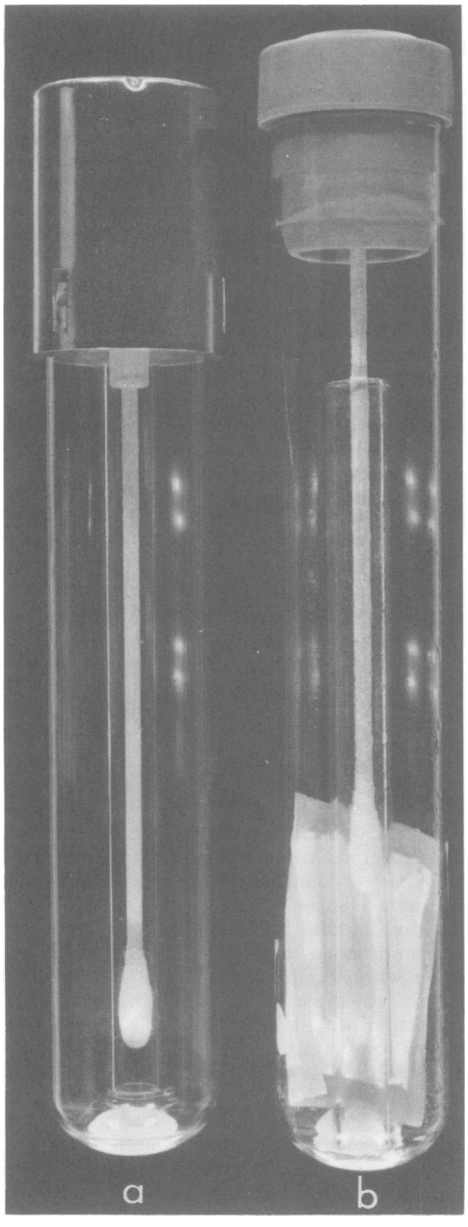

FIG. 1. *(a) Tube configuration for the inoculated swab used for AER and AGB storage, allowing interchange of gas through openings under the metal closure. (b) Storage configuration of the Anaerobic Specimen Collector used per instructions after inoculation of bacteria onto the swab.*

placed in the anaerobic glove box for anaerobic storage for at least 18 h before use.

Analysis of in vitro data. Each type of test consisting of timed exposures of a single species or of three- or five-part mixtures to the AER, AGB, and ATS environments was repeated on a separate occasion at least once. A log transformation of the viable counts before and after exposure was performed, and the results were expressed as a geometric mean of the percent survivors of the initial (zero-time) inoculum (Table 1). Use of percent survivors facilitates comparisons of viable counts after storage among species with slightly different initial counts on swabs. A minimum of 20 bacteria could be detected on a swab with this experimental protocol. On two occasions six swabs were consecutively charged with *E. coli* and *B. fragilis* and immediately quantitated as outlined above; this method of quantitation was demonstrated to be highly reproducible. On numerous occasions the initial mixed bacterial suspension was quantitated to enable comparison of the initial count with the count obtained from the zero-time, 0.1 ml-charged swabs after mixing with 1.9 ml of diluent. The average quantitative recovery of species from swabs was approximately 70% of the initial viable count.

Evaluation of clinical specimens. Clinical specimens were used for evaluation of the ATS if they: (i) were suspected of containing anaerobic bacteria, (ii) could be collected from body sites uncontaminated with normal flora, and (iii) had sufficient volume for charging test swabs. Fresh clinical material was immediately transported to the laboratory in an anaerobic transport device (Anaport, Anaswab; Scott Laboratories, Fiskeville, R.I.), in a syringe, or, infrequently, in an aerobic container when large volumes of pus were obtained (delivery within 15 min). The material was passed into the anaerobic glove box and thoroughly mixed on a Vortex mixer. ATS swabs were inoculated with 0.1-ml samples of the suspension outside the glove box (in air) as described for the in vitro-prepared mixtures. Swabs were immediately returned to the collector tubes and used per instructions. One swab was placed in the anaerobic glove box and immediately planted on media for the zero-time growth control. Other swabs were held in the ATS at room temperature in the dark for 2, 24, 48, and 72 h, after which time they were transferred to the anaerobic glove box, planted, and incubated at 37°C in a manner similar to that used for the original (zero-time) specimen. The test specimens were processed in a manner similar to that for routine specimens for aerobic and anaerobic cultures to see how the ATS would function in clinical use.

For inoculation of plated media, swabs were rolled over approximately one-fourth of the plate surface, and sequential quadrants were streaked with a bacteriological loop. All swabs were streaked onto plates in as identical a manner as possible so that each strain isolated could be semiquantitated, and growth occurring after the timed exposures could be compared with the original zero-time culture. The media used for anaerobic incubation were blood agar, phenylethyl alcohol agar, and kanamycin-vancomycin blood agar (kanamycin concentration, 75 μg/ml), all supplemented as described above. Aerobic media (placed in a CO_2 incubator) consisted of blood agar, phenylethyl alcohol agar or colistin-nalidixic acid blood agar, and MacConkey plates. After inoculation of plated media, the swab was placed in prereduced anaerobically sterilized chopped meat carbohydrate broth (Scott Laboratories) which was incubated anaerobically. Colonies on anaerobic plates were picked at 48 to 72 h, but plates were reexamined at intervals up to 7 days for emergence of slowly growing organisms. Aerobic plates

TABLE 1. *Survival of bacteria in pure culture and in polymicrobial mixtures on swabs stored in three environments*

Species	Time after inoculation (h)	% Survivors in pure culture[a]		% Survivors in three-part mixture			% Survivors in five-part mixture		
		ATS	AER	ATS	AGB	AER	ATS	AGB	AER
B. fragilis	2	1.42×10^2	1.33×10^2	1.60×10^2	2.95×10^2	1.31×10^2	3.46×10^2	5.20×10^2	1.61×10^2
	24	1.27×10^3	2.17×10^2	2.04×10^3	4.27×10^3	1.55×10^2	2.02×10^4	2.98×10^4	2.55
	48	3.1×10^3	1.05×10^2	4.32×10^3	1.06×10^4	1.74×10^2	2.58×10^4	3.65×10^4	0[b]
	72	3.19×10^3	5.3×10^1	2.46×10^3	9.44×10^3	1.66×10^2	2.10×10^4	2.78×10^4	0
P. anaerobius	2	1.68×10^2	1.01×10^2	1.29×10^2	3.13×10^2	1.05×10^2	1.91×10^2	3.63×10^2	7.24×10^1
	24	2.09×10^2	1×10^{-4}	2.06×10^3	3.27×10^3	2.32×10^1	1.54×10^3	2.08×10^2	4.19
	48	2.27×10^2	0	2.51×10^3	2.14×10^3	1.87×10^1	1.17×10^3	1.67×10^3	0
	72	1.95×10^2	0	1.82×10^3	3.59×10^3	1.18×10^{-1}	1.54×10^2	1.09×10^3	0
E. coli	2	2.19×10^2	2.35×10^2	2.40×10^2	1.0×10^3	2.60×10^2			
	24	4.04×10^3	3.54×10^4	2.29×10^3	2.11×10^3	2.37×10^4			
	48	3.56×10^3	3.05×10^4	1.93×10^3	2.32×10^3	1.86×10^4			
	72	3.0×10^3	3.08×10^4	1.51×10^3	2.66×10^3	2.16×10^4			
F. nucleatum	2	9.96×10^1	1.59×10^{-1}				9.89×10^1	1.71×10^2	1.49
	24	4.15×10^1	5×10^{-4}				1.72×10^2	4.10×10^2	0
	48	4.54	0				6.71×10^1	3.08×10^2	0
	72	6.0	0				1.95×10^1	1.53×10^2	0
S. epidermidis	2	1.12×10^2	1.14×10^2				1.47×10^2	3.11×10^2	1.59×10^2
	24	3.81×10^2	6.86×10^2				3.00×10^2	2.81×10^3	7.38×10^3
	48	5.81×10^2	1.04×10^3				1.91×10^2	3.83×10^3	2.64×10^3
	72	1.18×10^3	4.21×10^3				3.34×10^2	5.22×10^3	5.61×10^2
P. aeruginosa	2	1.78×10^2	1.92×10^2				1.59×10^2	9.27×10^2	2.67×10^2
	24	4.23×10^2	9.16×10^4				5.96×10^2	4.85×10^3	4.94×10^5
	48	4.14×10^2	1.71×10^5				8.41×10^2	7.20×10^3	8.43×10^5
	72	3.94×10^2	3.13×10^5				4.26×10^2	7.31×10^3	9.78×10^5

[a] % Survivors = (bacterial count after storage/initial count) × 100. Initial (zero time) viable count considered to be 100% (10^2).

[b] Limit of detection on swabs was 20 bacteria.

were usually examined and picked at 24 h, but were held for 4 days.

The types and numbers of bacteria isolated after storage in the ATS were compared with those obtained from the zero-time culture. The amount of growth of each strain on plates was recorded by using 1+ for growth only within the primary zone of inoculation and 2+, 3+, and 4+ for growth in each zone sequentially streaked, respectively. Anaerobic organisms were identified by Gram stain morphology, gas liquid chromatography, and biochemical testing (8). Aerobic and facultative organisms were identified by using standard laboratory procedures (11).

RESULTS

Three-part mixture of bacteria. The polymicrobial mixture consisting of *B. fragilis, P. anaerobius,* and *E. coli* was exposed on swabs to the ATS, AGB, and AER environments for 2, 24, 48, and 72 h. All species remained viable in the ATS and AGB environments for 72 h, and the viable count of each organism increased more than 1 log ($\log_{10}$) by 24 h and remained high for 72 h (Table 1). In the AER environment the number of *P. anaerobius* decreased by 3 logs in 72 h. Interestingly, *B. fragilis* survived in the AER environment, with little change from the initial bacterial count in 72 h. The number of *E. coli* increased by approximately 2 logs by 24 h, and this count persisted throughout 72 h in the AER environment. When the survival of each species exposed to the ATS and AER environments in the three-part mixture was compared with its survival when exposed in pure culture, *B. fragilis* and *E. coli* responded similarly, but *P. anaerobius* had higher viable counts in the three-part mixture than when exposed in pure culture, particularly in the AER environment (Table 1).

Over the 72-h exposure period the ATS environment maintained the three species of bacteria in approximately the same proportions as found in the original inoculum (Fig. 2). Results were similar for the AGB environment. In contrast, the AER environment was less effective in maintaining proportionality among strains, and a wide disparity in the number of survivors of each strain was observed at 24, 48, and 72 h (Fig. 3).

Five-part mixture of bacteria. A polymicrobial mixture consisting of *B. fragilis, P. anaerobius, F. nucleatum, S. epidermidis,* and *P. aeruginosa* was exposed to the different environments as just described for the three-part mixture. However, *B. fragilis* and *P. anaerobius* responded differently to exposure in the five-part mixture than they did in the three-part mixture. When these organisms were exposed to the AER environment in the five-part mixture,

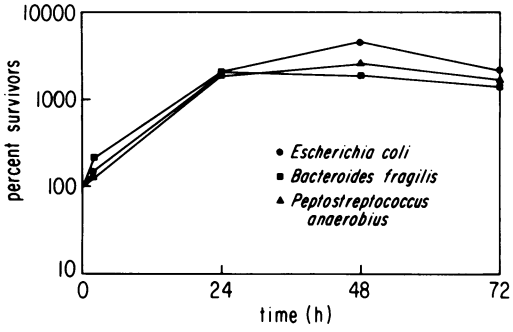

FIG. 2. *Percent survivors of anaerobic and facultative bacteria in a polymicrobial mixture stored on swabs in the Anaerobic Specimen Collector for 72 h.*

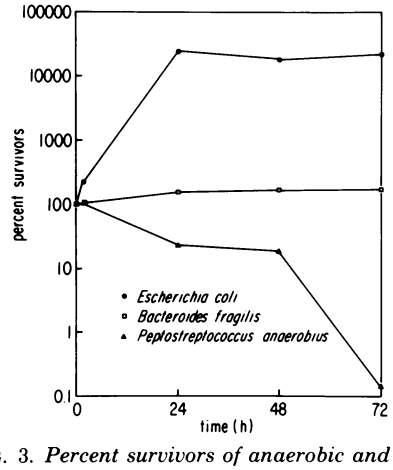

FIG. 3. *Percent survivors of anaerobic and facultative bacteria in a polymicrobial mixture stored on swabs in AER conditions for 72 h.*

the viable counts of *B. fragilis* and *P. anaerobius* decreased more than 1 log by 24 h, and no organisms were detected at 48 and 72 h (Table 1). In contrast, after exposure to the AER environment in the three-part mixture, the viable count of *B. fragilis* remained approximately the same throughout the 72 h, and *P. anaerobius* decreased in count by approximately 1 log in 48 h and was detectable at 72 h. When exposed to ATS and AGB environments in the five-part mixture, *B. fragilis* and *P. anaerobius* increased in count by 2 and 1 log, respectively, and counts remained higher than those in the original inoculum for 72 h (Table 1).

F. nucleatum survived in the ATS with less than a 1-log decrease by 72 h. In the AGB viable counts remained slightly above the initial count throughout 72 h, whereas *F. nucleatum* was rapidly inactivated in the AER environment, decreasing by approximately 2 logs in 2 h and undetectable by 24 h (Table 1). There was no substantial difference in the response of the facultative organism, *S. epidermidis,* in the three different environments, and counts were similar to or 1 log greater (at 72 h) than those present on the swab initially (Table 1). The aerobic organism, *P. aeruginosa,* demonstrated a very different pattern, with an increase in viable count of more than 3 logs by 24 h and approximately 4 logs by 72 h in the AER environment. In the ATS environment the number of *P. aeruginosa* increased only slightly, and counts remained fairly constant through 72 h, whereas in the AGB environment viable counts were similar to but somewhat higher than those in the ATS environment.

The response of *P. anaerobius, F. nucleatum, S. epidermidis,* and *P. aeruginosa* to the ATS and AER environments was similar whether the organisms were exposed in pure culture or in the five-part mixture, except that counts were slightly higher, in general, for *P. anaerobius* and *F. nucleatum* when exposed in the mixture. The response of *B. fragilis* was distinctly different when it was exposed in the five-part mixture compared with exposure in pure culture (Table 1).

Changes in relative proportions among various species with time were least in the ATS environment (Fig. 4), but results in the AGB environment were similar. In the AER environment the anaerobic species rapidly disappeared, whereas the facultative and aerobic organisms markedly increased in numbers (Fig. 5), resulting in drastic alterations in relative proportions among species.

Clinical specimens. Samples from 16 clinical

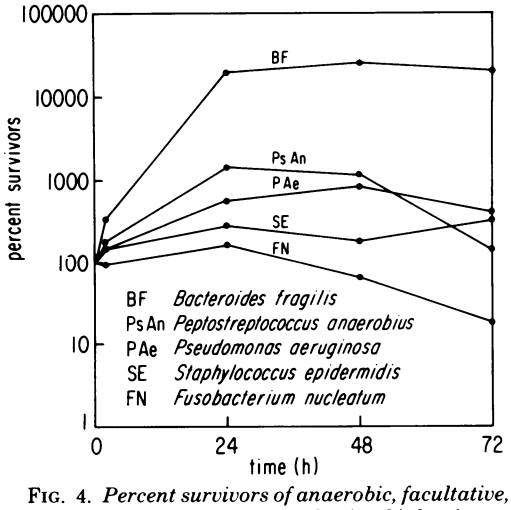

FIG. 4. *Percent survivors of anaerobic, facultative, and aerobic bacteria in a polymicrobial mixture stored on swabs in the Anaerobic Specimen Collector for 72 h.*

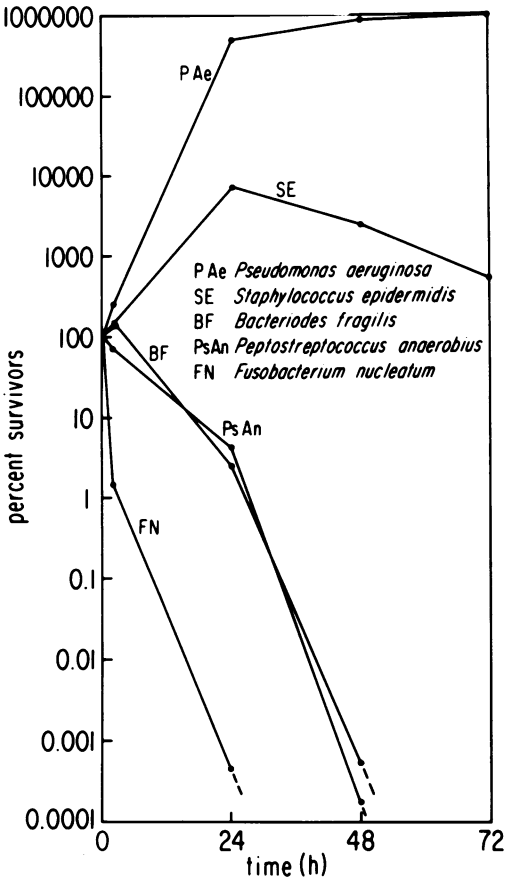

FIG. 5. *Percent survivors of anaerobic, facultative, and aerobic bacteria in a polymicrobial mixture stored on swabs in AER conditions for 72 h.*

specimens were placed in the ATS environment, and the number and type of species present after 2, 24, 48, and 72 h were compared with the species initially (at zero time) isolated from each specimen. Specimens were obtained from the following sources: pelvic abscess (three), Bartholin abscess (three), subphrenic abscess (two), wound (two), and one each from thoracentesis fluid, empyema, peritoneal fluid, gall bladder, amniotic fluid, and pancreatic cyst. Some representative examples to demonstrate the various effects of storage of clinical specimens in the ATS are given in Table 2. Of the 16 specimens, 5 contained only aerobic organisms, and 7 contained only anaerobic or microaerophilic bacteria, whereas 4 specimens had mixed aerotolerance types. Six specimens contained only a single species; three were aerobic, and three were anaerobic. A total of 18 aerobic species and 34 anaerobic and microaerophilic species were isolated. The semiquantitative estimate of growth of each species after the different exposure periods indicated that 12 species increased, 7 species decreased, and 8 species initially increased and then subsequently decreased over the 72-h exposure period. Most often the change was only one unit (i.e., 2+ growth to 3+ growth), which might not always accurately reflect an actual quantitative difference because of some unavoidable variation in streaking the plates. However, there was normally a consistent and reasonable pattern in the amount of growth throughout the exposure periods tested. The remaining 25 species appeared numerically stable throughout the 72 h. Bacteria that increased in growth by 2 units during this period were generally facultative gram-negative bacilli (such as *E. coli*) and *Bacteroides* species. Only 6 (4 anaerobic and 2 microaerophilic species) of the 52 species isolated were lost during the storage period, 3 by 24 h and 3 more by 72 h. In one instance a *Streptococcus faecalis* which was not isolated initially or at 2 h was isolated at 24, 48, and 72 h, probably reflecting growth of the organism, which was originally present in low numbers. A group D *Streptococcus* present in a gall bladder specimen demonstrated the same amount of growth after each storage period through 72 h, but at 48 and 72 h it was only found on the anaerobic plates and was not observed on aerobically incubated media.

DISCUSSION

For testing the various storage environments with in vitro-prepared mixtures of bacteria, increased test sensitivity was obtained by using bacteria in log phase and inoculating these onto swabs so that the organisms were more directly exposed to ambient environmental conditions (i.e., drying, oxygen) than if contained in liquid or tissue samples. The inocula for charging the swabs were prepared in nutrient broth because clinical material normally contains nutrients (present in pus, blood, serous material, and other body fluids). Under these test conditions aerobic, facultative, and anaerobic bacteria in pure culture and in polymicrobial mixtures survived in the ATS environment for 72 h. Survival was similar to that demonstrated in an earlier test of the prototype model of the ATS (14). The viable count of most species increased during the storage time, and *F. nucleatum* was the only organism to drop below (approximately 1 log) the initial count by 72 h. Results in the AGB environment were similar, except that counts of most species were slightly higher than those in the ATS environment. In the AER environment the aerobic and facultative bacteria increased in count during the 72 h, but anaerobic bacteria

TABLE 2. *Effect of storage in anaerobic specimen collector on bacteria present in clinical specimens*

Type of specimen	Organisms isolated at zero time	Amt of growth on plates after storage for:[a]				
		0 h	2 h	24 h	48 h	72 h
Peritoneal fluid	*Candida albicans*	1+	1+	1+	1+	2+
Empyema	Coagulase-negative *Staphylococcus*	4+	2+	2+	2+	2+
	P. aeruginosa	4+	4+	4+	4+	4+
	E. coli	4+	4+	4+	4+	4+
	B. fragilis	4+	4+	4+	4+	4+
	Bacteroides species	4+	4+	4+	4+	4+
	Propionibacterium avidium	4+	4+	4+	4+	4+
	Lactobacillus jensenii	4+	4+	4+	4+	4+
	Peptococcus magnus	4+	4+	4+	4+	4+
Bartholin gland abscess	*Bacteroides bivius*	4+	4+	4+	4+	4+
Pelvic abscess	*Streptococcus constellatus*	4+	2+	2+	1+	1+
	P. anaerobius	4+	4+	4+	4+	4+
	Clostridium clostridiiforme	4+	4+	4+	4+	4+
	Bacteroides melaninogenicus subsp. *intermedius*	3+	3+	3+	4+	4+
	F. nucleatum	3+	3+	3+	3+	3+
	Bacteroides thetaiotaomicron	1+	1+	1+	1+	1+
Subphrenic abscess	*E. coli*	2+	3+	4+	4+	4+
	Streptococcus faecalis	2+	2+	2+	2+	3+
Cul-de-sac abscess	*Actinomyces israelii*	4+	4+	3+	3+	1+
	Bacteroides thetaiotaomicron	2+	2+	3+	4+	4+
	F. nucleatum	2+	2+	3+	2+	2+
	Eubacterium species	2+	2+	3+	3+	2+
	Bacteroides species	2+	2+	3+	2+	0
Bartholin gland abscess	*E. coli*	4+	4+	4+	4+	4+
Subphrenic-perihepatic abscess	*Streptococcus intermedius*	3+	3+	2+	3+	3+
	B. fragilis	3+	4+	4+	4+	4+
	F. nucleatum	3+	3+	3+	2+	2+
	Peptostreptococcus micros	3+	2+	0	0	0
Pelvic abscess	*Peptococcus asaccharolyticus*	3+	3+	2+	4+	3+

[a] Amount of growth: 1+, growth in primary zone of streak only; 2+, 3+, and 4+, growth in second-, third-, and fourth-streaked quadrants on plate, respectively; 4+, highest number of organisms.

were rapidly inactivated, except for *B. fragilis* exposed in pure culture or in the three-part mixture. The basis for the rapid inactivation of *B. fragilis* in the five-part mixture under AER conditions is unknown, although an antagonistic compound produced by *P. aeruginosa* growing under aerobic conditions is a possibility. Prolonged survival of pure cultures of *B. fragilis* in aerobic conditions has been demonstrated by others (3, 4, 13, 14).

In addition to assuring survival of bacteria, an adequate transport system should maintain organisms in polymicrobial mixtures in their original proportions over the required storage period. The presence of nutrients tends to select for the more rapidly growing organisms, but the gaseous environment also regulates growth, as demonstrated by the minimal change in viable count of *P. aeruginosa* in the ATS in contrast to its rapid growth in the AER environment. Positive and negative interactions among species in a mixture may be important, as illustrated by the apparent protective effect (probably by *E. coli*) of *P. anaerobius* in the three-part mixture and the rapid inactivation of *B. fragilis* in the five-part mixture. Pretreatment of patients with antibiotics before collection of a bacteriological specimen may also affect members of a mixed population differently during storage. Of the storage environments tested, the ATS environment was generally most efficient at holding the count of each species closest to the number present initially. The AGB environment was only slightly less effective in this regard, whereas relative proportions were rapidly altered in the AER environment, particularly with the five-part mixture. However, the quantitative data from the five-part mixture emphasizes that even when an effective anaerobic environment is used (ATS or AGB), alterations in relative proportions of species do occur and increase with storage time, so that, optimally, clinical specimens

should be cultured on plates at the earliest possible time. These data support the use of anaerobic transport for aerobic and facultative species as well as anaerobes, particularly when a delay in culture of a polymicrobial specimen is anticipated. Use of anaerobic transport for aerobic and facultative bacteria has been suggested by other authors, based on testing of pure cultures (3, 5, 14, 15).

Survival and maintenance of original proportions of anaerobic bacteria were generally better in the ATS environment than with certain other transport systems tested previously (1, 4, 7, 15), although direct comparisons of identical species in different studies cannot be made due to possible variations in oxygen tolerance and other growth characteristics among different strains of a species. The ATS provides a moist anaerobic environment, whereas survival on swabs within dry gassed-out tubes is poor (1, 15). Generally, good results were obtained with pure cultures (3) or aspirated clinical specimens (7) stored in gassed-out transport vials or in a capped syringe. Although survival of anaerobic bacteria in transport media has often been inadequate, certain transport media prepared under anaerobic conditions successfully maintained viability of fresh human plaque flora (12) and pure cultures of anaerobes (E. Mena, V. P. Dowell, Jr., F. S. Thompson, and A. Y. Armfield, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C198, p. 68). However, the tests with human plaque flora enumerated only overall numbers and did not investigate changes in relative proportions of the organisms in mixed culture. Most anaerobes could be recovered after 24 h of aerobic storage in at least 2 ml of purulent specimen from mixed infection (2), but 4 of 26 anaerobes originally present were lost and 5 other anaerobic species decreased significantly in viable count. Although in certain specimens the presence of purulent exudate and aerobic and facultative organisms may protect many anaerobic bacteria, often a minimal volume of aspirated material is available, material is collected on swabs, transport is delayed longer than anticipated, species much less aerotolerant than *B. fragilis* are present, and aerobic and facultative organisms may not be present. For these reasons, routine provision of an adequate anaerobic transport system to handle all types of specimens is probably desirable.

Although tests with the ATS using in vitro-prepared mixtures had indicated that organisms would survive for 72 h, the ATS was also tested with fresh clinical material in case the oxygen tolerances and other characteristics of freshly isolated organisms were different from laboratory cultures. Survival of aerobic, facultative, and anaerobic bacteria from clinical material was generally excellent in the ATS for 72 h, and the pattern of recovery of isolates after each storage period was quite regular. The problem of alteration of bacterial populations during storage time, however, needs to be solved before the ATS or any other efficient anaerobic transport system can be used for extended storage periods. Selective media would probably aid recovery from clinical material when changes in counts among species occurred, but the information on organisms actually predominant in infection would be lost. This problem has been documented by others with regard to mixed cultures containing anaerobes (2, 7) or when only aerobic and facultative bacteria were cultured (9). Although refrigeration of specimens has been suggested as a possible method to slow or halt changes in bacterial concentrations, it has been controversial, and little data has been available. *B. fragilis* was reported to be inactivated by chilling (6), but recent data with an anaerobic transport medium demonstrated good survival of pure cultures of *B. fragilis* and other anaerobes at 4°C for 48 h (Mena et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C198, p. 68). Tests with the ATS or other effective transport devices on the effects of refrigeration on maintenance of original proportions of mixed bacterial populations would be useful.

In conclusion, the present studies have demonstrated that the ATS would be an excellent transport container for delivery of specimens suspected of containing anaerobes to microbiology laboratories. Although survival of pure cultures of anaerobes for 72 h and perhaps longer could be expected in the ATS, these experiments also indicated that lengthy storage of polymicrobial specimens may sometimes alter the bacteriological findings. This problem doubtlessly is common to all transport devices currently available and appears to be minimized by use of an efficient anaerobic container such as the ATS. Optimal results are certainly afforded by immediate culture of polymicrobial clinical specimens, but these data suggest that storage in the ATS for periods less than 12 h would give acceptable culture results, particularly with the use of selective media. For laboratories that close or provide no anaerobic planting service at night, use of the ATS (or other effective anaerobic system) would avoid the necessity of calling a night call technologist or planting of clinical specimens by busy clinicians (10).

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