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Survival of Anaerobic and Aerobic Bacteria in a Nonsupportive Gassed Transport System

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Survival of anaerobic and aerobic bacteria in a commercially available, nonsupportive, gassed (oxygen-free) transport container (Anaport) was evaluated quantitatively. Saline-suspended obligate anaerobes survived significantly better in the gassed container than in aerobic control tubes ($P < 0.025$, t test), and counts were virtually unchanged after 8 h of holding. Similarly, initial counts and relative proportions of a mixture of *Bacteroides fragilis* and *Staphylococcus aureus* were maintained for 72 h. The value of the gassed transport system was less apparent when microorganisms were suspended in nutrient broth. The major advantage of the gassed transport system appears to be for holding of specimens collected by saline irrigation.

Appropriate collection and transport of specimens is an important prerequisite for the successful isolation of fastidious anaerobes in the diagnosis of anaerobic infections (1). For this purpose, the gassed (oxygen-free) non-nutrient container has been considered an optimal transport system (2, 3) and is commercially available for general use. Despite this, there have been little published data to examine the survival of various anaerobic and aerobic bacteria in such a system and the maximum interval during which storage of specimens in such a system may be beneficial. This study was undertaken to evaluate quantitatively the survival of various anaerobes and aerobes in a commercially available gassed transport system (Anaport, Scott Laboratories, Fiskeville, R.I.) as compared to holding under aerobic conditions.

MATERIALS AND METHODS

Test organisms. Bacteria chosen in this study were representative of clinical isolates with varying degrees of fastidiousness and aerotolerance. These included six obligate anaerobes *(Bacteroides fragilis, Peptostreptococcus intermedius, Veillonella parvula, Clostridium perfringens, Propionibacterium acnes, Eubacterium lentum),* six facultative bacteria *(Escherichia coli, Staphylococcus aureus, Streptococcus faecalis,* group A *Streptococcus, Haemophilus influenzae, Neisseria gonorrhoeae),* and one aerobe *(Pseudomonas aeruginosa).* All were stock cultures of clinical isolates maintained either by lyophilization or in 20% skim milk at −70 C until ready for study.

Transport systems. The gassed non-nutrient transport system evaluated (Anaport, Scott Laboratories) consisted of stoppered 10-ml vials evacuated and replaced with oxygen-free CO_2, moistened for autoclave sterilization with 0.1 ml of Virginia Polytechnic Institute (VPI) salts solution (3), and containing a resazurin indicator (Eh = −42 mV). All gassed containers studied were from the same commercial batch. Sterile, aerobic, screw-cap glass tubes (13 by 100 mm) (Kimax, Kimble, Toledo, Ohio) were used for controls.

Culture, dilution, and quantitation. Stock cultures of obligate anaerobes were grown in prereduced, anaerobically sterilized, thioglycolate broth (Difco), streaked on brain heart infusion agar (Difco) enriched with 5% sheep blood, and incubated in a GasPak anaerobic jar evacuated and replaced with a gas mixture of 80% N_2, 10% H_2, and 10% CO_2. Colonies were washed off, suspended in sterile, physiological saline without bacteriostatic preservative, and adjusted to a turbidity corresponding to a MacFarland no. 1/2 nephalometer standard. Aliquots (5 ml) of this saline suspension were transferred by needle and syringe into a gassed vial and a corresponding aerobic control tube and kept at room temperature. Immediately (0 h) and serially at 1, 2, 4, 8, 24, 48, and 72 h, aliquots of 0.5 ml were removed from each system with a needle and syringe. Colony counts of viable organisms were determined anaerobically and in triplicate in roll tubes containing prereduced, anaerobically sterilized brain heart infusion agar (Difco) supplemented with cysteine, yeast extract, and menadione according to the VPI *Anaerobe Laboratory Manual* (3).

Stock cultures of facultative and aerobic bacteria were grown in Mueller-Hinton broth (BBL) and streaked aerobically on brain heart infusion blood agar. Colonies were washed off, suspended, and diluted in sterile physiological saline without bacteriostatic preservative and transferred to the gassed

vials and aerobic control tubes in the same manner as with obligate anaerobes. Colony counts of facultative and aerobic bacteria were likewise determined serially and in triplicate on brain heart infusion blood agar plates, using a 0.01-ml platinum quantitative loop (Scientific Products, McGaw Park, Ill.).

In a separate experiment, survival curves were similarly determined in gassed vials and aerobic control tubes, with the test organisms suspended either in thioglycolate broth (obligate anaerobes) or Mueller-Hinton broth (aerobic and facultative bacteria) rather than in physiological saline.

Finally, to determine the survival of anaerobic and aerobic components of a mixed flora, saline and broth suspensions of *B. fragilis* and *S. aureus* were admixtured in equal proportions (vol/vol) prior to instillation in the gassed transport system. Serial colony counts of both organisms were carried out in triplicate in roll tubes containing prereduced, anaerobically sterilized brain heart infusion agar as described earlier.

RESULTS

Colony counts of saline-suspended anaerobic and aerobic bacteria surviving at various intervals in gassed containers and their corresponding aerobic control tubes are summarized in Table 1. Obligate anaerobes held under aerobic conditions displayed varying degrees of aerotolerance. A 3-log decrease in colony counts occurred after a mean interval of 8.7 h. *E. lentum* was most sensitive, with a 3-log decrease in 1.5 h, and none could be recovered in 4 h. *P. intermedius, V. parvula,* and *B. fragilis* were intermediately sensitive, with a 3-log decrease in 3, 5, and 4 h, respectively, and none could be recovered between 8 and 24 h. *Propionibacterium acnes* and *C. perfringens* were least sensitive, with a 3-log decrease in 10 and 29 h, respectively, and none could be recovered between 48 and 72 h. All obligate anaerobes survived better in gassed containers than in aerobic control tubes, with significant differences in counts ($P < 0.025$, t test) by 2 h and thereafter. Except for *E. lentum,* colony counts of oligate anaerobes in gassed containers were virtually unchanged after 8 h. The mean interval for a 3-log decrease with these organisms was 53 h, compared to 8.7 h in aerobic control tubes ($P < 0.0025$, t test). *E. lentum* survived better in the gassed container ($P < 0.0025$, t test) at 2 and 4 h; none could be recovered by 8 h.

Similar to obligate anaerobes, both *Escherichia coli* and *Pseudomonas aeruginosa* declined in colony counts under aerobic conditions, with a 3-log decrease after 4 and 8 h, respectively (Table 1). However, in contrast to obligate anaerobes, significant counts of these aerobes remained after 48 h. Of interest was the finding that both aerobes suspended in saline also survived better in the gassed container ($P < 0.025$, t test) by 1 h and thereafter.

Survival of anaerobic and aerobic bacteria suspended in nutrient broth in gassed containers and their corresponding aerobic control tubes is shown in Table 2. Surprisingly, when suspended in broth, both *P. intermedius* and *B. fragilis* survived well under aerobic conditions even at 72 h, and their colony counts were virtually identical to those in gassed containers. Among the aerobic organisms, with the exception of *N. gonorrhoeae* and *H. influenzae,* bacterial counts increased serially in both transport systems. *N. gonorrhoeae* and *H. influenzae* maintained their initial counts for 24 h, but rapidly declined in number thereafter.

Both *B. fragilis* and *S. aureus,* mixed in saline suspension and held in the nonsupportive gassed transport system, maintained their initial counts and relative proportions and survived significantly better than in aerobic control tubes ($P < 0.0005$, t test) at 72 h (Table 3). In contrast, colony counts of *B. fragilis* and *S. aureus* increased steadily in both gassed and control containers when mixed in nutrient broth and decreased steadily in aerobic control tubes when mixed in saline. Surprisingly, a saline suspension of *B. fragilis* mixed with *S. aureus* was more aerotolerant under aerobic conditions than a saline suspension of *B. fragilis* in pure culture, and significant viable counts remained at 72 h in the aerobic control tube.

DISCUSSION

Appropriate transportation and minimal delay in processing of clinical specimens are important prerequisites for the successful isolation of fastidious obligate anaerobes (1, 2). An ideal transport system should be nonselective as well as nonsupportive, capable of maintaining viability without promoting growth or appreciably altering the relative proportion of various organisms initially present in the clinical specimen. Our data of survival of saline-suspended obligate anaerobes clearly demonstrate the detrimental effect of oxygen and the protective effect of the gassed transport system. All obligate anaerobes tested survived better in the gassed transport system, and, except for *E. lentum,* colony counts were virtually unchanged after 8 h. In addition, the gassed container maintained the initial counts and their relative proportions of both *B. fragilis* and *S. aureus* after 72 h when mixed in saline suspension. A surprising finding was that saline sus-

TABLE 1. *Survival of saline-suspended anaerobic and aerobic bacteria in gassed and aerobic control tubes at different time periods*[a]

Organism	Tube	Time (h)							
		0	1	2	4	8	24	48	72
Eubacterium lentum	Gassed	6.3 ± 0.06	4.7 ± 0.08	4.0 ± 0.01[b]	2.5 ± 0.09[c]	0	0	0	0
	Aerobic	6.7 ± 0.01	5.6 ± 0.25	1.7 ± 0.59	0	0	0	0	0
Peptostreptococcus intermedius	Gassed	7.7 ± 0.26	7.4 ± 0.04[b]	7.4 ± 0.02[b]	7.7 ± 0.13[c]	7.4 ± 0.03[c]	7.0 ± 0.10[c]	6.1 ± 0.04[c]	4.4 ± 0.10[c]
	Aerobic	7.4 ± 0.05	6.2 ± 0.30	5.5 ± 0.55	3.6 ± 0.08	0	0	0	0
Veillonella parvula	Gassed	7.4 ± 0.06	7.3 ± 0.09	7.3 ± 0.05[d]	7.1 ± 0.04[c]	7.0 ± 0.05[c]	6.7 ± 0.02[c]	5.9 ± 0.15[c]	4.2 ± 0.06[c]
	Aerobic	7.2 ± 0.17	7.0 ± 0.18	6.4 ± 0.43	4.9 ± 0.16	1.9 ± 0.14	0	0	0
Bacteroides fragilis	Gassed	6.4 ± 0.07	6.2 ± 0.19[b]	6.4 ± 0.20[c]	5.4 ± 0.24[c]	5.2 ± 0.33[c]	5.2 ± 0.23[c]	4.6 ± 0.76[c]	3.2 ± 0.79[c]
	Aerobic	6.2 ± 0.29	5.3 ± 0.06	4.6 ± 0.10	3.0 ± 0.06	1.8 ± 0.23	0	0	0
Propionibacterium acnes	Gassed	8.5 ± 0.07	8.3 ± 0.04[d]	8.4 ± 0.16[d]	8.2 ± 0.06[b]	8.5 ± 0.04[c]	8.3 ± 0.11[c]	7.4 ± 0.28[c]	6.9 ± 0.09[c]
	Aerobic	8.4 ± 0.15	8.2 ± 0.05	7.9 ± 0.21	6.8 ± 0.33	6.1 ± 0.17	3.8 ± 0.16	0	0
Clostridium perfringens	Gassed	7.5 ± 0.10	7.4 ± 0.01[d]	7.4 ± 0.06[d]	7.3 ± 0.02[d]	7.2 ± 0.06[b]	6.1 ± 0.18[b]	3.4 ± 0.07[b]	1.2 ± 0.17[c]
	Aerobic	7.4 ± 0.02	7.3 ± 0.04	7.2 ± 0.03	7.1 ± 0.15	6.5 ± 0.09	5.1 ± 0.17	1.2 ± 0.46	0
Escherichia coli	Gassed	8.2 ± 0.15	8.1 ± 0.01[c]	7.6 ± 0.07[c]	8.0 ± 0.14[c]	7.5 ± 0.21[c]	7.5 ± 0.05[c]	7.1 ± 0.16[c]	6.9 ± 0.08[c]
	Aerobic	8.2 ± 0.06	6.4 ± 0.05	5.8 ± 0.08	5.2 ± 0.17	4.3 ± 0.35	1.6 ± 0.05	2.3 ± 0.30	0
Pseudomonas aeruginosa	Gassed	7.9 ± 0.05	7.6 ± 0.16[d]	7.9 ± 0.14[c]	7.5 ± 0.27[c]	7.4 ± 0.01[c]	7.0 ± 0.14[c]	6.2 ± 0.04[c]	5.7 ± 0.16[b]
	Aerobic	8.1 ± 0.3	7.3 ± 0.07	6.1 ± 0.07	5.6 ± 0.12	5.1 ± 0.05	4.3 ± 0.18	3.4 ± 0.13	2.6 ± 0.67

[a] Expressed as mean $\log_{10}$ number of bacteria per milliliter ± standard deviation of triplicates.
[b] Significant difference compared with aerobic control tubes; $P < 0.005$, t test analysis.
[c] Significant difference compared with aerobic control tubes; $P < 0.0005$, t test analysis.
[d] Significant difference compared with aerobic control tubes; $P < 0.05$, t test analysis.

TABLE 2. *Survival of broth-suspended anaerobic and aerobic bacteria in gassed and aerobic control tubes at different time periods*[a]

Organism	Tube	Time (h)							
		0	1	2	4	8	24	48	72
Peptostreptococcus intermedius	Gassed	6.8 ± 0.20	6.8 ± 0.03	6.9 ± 0.19	6.8 ± 0.04	6.7 ± 0.02	6.7 ± 0.10	5.0 ± 0.15	5.6 ± 0.27
	Aerobic	7.0 ± 0.10	6.8 ± 0.05	6.9 ± 0.08	7.0 ± 0.05	7.0 ± 0.12	6.9 ± 0.05	6.7 ± 0.11	6.7 ± 0.04
Bacteroides fragilis	Gassed	8.3 ± 0.05	8.5 ± 0.06	8.3 ± 0.02	8.4 ± 0.06	8.3 ± 0.08	8.5 ± 0.10	8.3 ± 0.09	7.7 ± 0.01
	Aerobic	8.4 ± 0.07	8.4 ± 0	7.7 ± 0.26	8.5 ± 0.05	8.5 ± 0.10	8.7 ± 0.29	8.8 ± 0.13	8.1 ± 0.07
Staphylococcus aureus	Gassed	7.7 ± 0.07	7.7 ± 0.19	7.6 ± 0.31	8.2 ± 0.18	8.4 ± 0.26	8.4 ± 0.13	8.8 ± 0.29	8.8 ± 0.33
	Aerobic	7.3 ± 0.74	7.7 ± 0.16	8.1 ± 0.14	8.5 ± 0.25	9.0 ± 0.12	9.6 ± 0.09	9.5 ± 0.09	9.6 ± 0.19
Group A *Streptococcus*	Gassed	6.6 ± 0.59	7.1 ± 0.33	7.2 ± 0.27	7.1 ± 0.45	6.8 ± 0.30	8.1 ± 0.10	8.5 ± 0.03	7.8 ± 0.47
	Aerobic	6.7 ± 0.72	7.3 ± 0.31	8.1 ± 0.16	7.6 ± 0.35	8.4 ± 0.70	8.5 ± 0.10	8.2 ± 0.13	8.5 ± 0.12
Streptococcus faecalis	Gassed	7.8 ± 0.12	7.8 ± 0.12	8.0 ± 0.09	8.2 ± 0.19	8.8 ± 0.28	8.7 ± 0.24	9.0 ± 0.03	8.9 ± 0.28
	Aerobic	7.6 ± 0.05	7.9 ± 0.30	8.1 ± 0.04	8.8 ± 0.28	8.9 ± 0.19	9.2 ± 0.25	8.7 ± 0.77	9.0 ± 0.14
Escherichia coli	Gassed	7.2 ± 0.46	7.3 ± 0.19	7.2 ± 0.16	7.6 ± 0.23	8.0 ± 0.10	8.4 ± 0.38	8.1 ± 0.18	8.2 ± 0.14
	Aerobic	7.2 ± 0.23	7.7 ± 0.18	7.2 ± 0.20	8.1 ± 0.02	8.8 ± 0.40	8.9 ± 0.20	8.8 ± 0.72	9.2 ± 0.23
Pseudomonas aeruginosa	Gassed	7.2 ± 0.29	6.9 ± 0.26	7.5 ± 0.23	7.2 ± 0.23	7.5 ± 0.11	7.7 ± 0.48	7.9 ± 0.26	8.6 ± 0.33
	Aerobic	7.2 ± 0.14	7.5 ± 0.16	7.7 ± 0.28	7.9 ± 0.24	8.3 ± 0.06	9.0 ± 0.14	9.8 ± 0.11	9.9 ± 0.07
Neisseria gonorrhoeae	Gassed	8.3 ± 0.13	8.4 ± 0.11	8.4 ± 0.06	8.5 ± 0.04	8.3 ± 0.24	7.6 ± 0.17	4.9 ± 0.16	0
	Aerobic	8.4 ± 0.43	8.4 ± 0.11	8.4 ± 0.06	8.5 ± 0.08	8.0 ± 0.21	6.7 ± 0.29	2.2 ± 0.34	0
Haemophilus influenzae	Gassed	8.0 ± 0.03	7.8 ± 0.20	7.5 ± 0.22	7.6 ± 0.32	7.3 ± 0.18	7.2 ± 0.30	5.8 ± 0.12	2.4 ± 0.45
	Aerobic	8.0 ± 0.17	7.6 ± 0.28	7.7 ± 0.14	8.1 ± 0.17	8.7 ± 0.12	8.9 ± 0.22	6.2 ± 0.22	3.1 ± 0.07

[a] Expressed as mean $\log_{10}$ number of bacteria per milliliter ± standard deviation of triplicates.

TABLE 3. *Survival of B. fragilis-S. aureus admixture in gassed and aerobic control tubes at different time periods*[a]

Admixture	Tube	Time (h)							
		0	1	2	4	8	24	48	72
Saline suspension									
B. fragilis	Gassed	6.8 ± 0.10	7.0 ± 0.07	6.5 ± 0.01	6.5 ± 0.02	6.5 ± 0.10	6.7 ± 0.13	6.7 ± 0.05	6.7 ± 0.14[b]
	Aerobic	6.9 ± 0.10	7.0 ± 0.14	6.8 ± 0.07	6.8 ± 0.21	6.9 ± 0.13	6.9 ± 0.21	6.9 ± 0.45	5.8 ± 0.10
S. aureus	Gassed	7.7 ± 0.17	7.6 ± 0.19	7.2 ± 0.07	7.3 ± 0.11	7.7 ± 0.01	7.5 ± 0.05	7.6 ± 0.09	8.1 ± 0.10[b]
	Aerobic	8.1 ± 0.17	8.1 ± 0.24	7.9 ± 0.13	7.7 ± 0.04	7.8 ± 0.06	7.6 ± 0.07	7.3 ± 0.13	6.1 ± 0.17
Broth suspension									
B. fragilis	Gassed	6.4 ± 0.05		6.6 ± 0.11	6.4 ± 0.11	6.9 ± 0.08	7.1 ± 0.09	7.2 ± 0.04	7.0 ± 0.01[c]
	Aerobic	6.3 ± 0.10		6.7 ± 0.18	6.8 ± 0.04	7.0 ± 0.15	7.3 ± 0.01	7.1 ± 0.04	6.7 ± 0.17
S. aureus	Gassed	7.1 ± 0.10		7.1 ± 0.06	7.6 ± 0.05	8.1 ± 0.22	8.4 ± 0.20	8.5 ± 0.01	8.2 ± 0.13[b]
	Aerobic	7.0 ± 0.10		7.6 ± 0.09	8.0 ± 0.09	8.8 ± 0.06	8.8 ± 0.17	8.4 ± 0.20	7.6 ± 0.07

[a] Expressed as mean $\log_{10}$ bacteria per milliliter ± standard deviation of triplicates.
[b] Significant difference compared with aerobic control tubes; $P < 0.0005$, *t* test analysis.
[c] Significant difference compared with aerobic control tubes; $P < 0.05$, *t* test analysis.

pensions of both *Escherichia coli* and *Pseudomonas aeruginosa* also survived better in the gassed transport vial but declined in counts under aerobic conditions. Sterile saline may be toxic to some bacteria, whereas balanced salt solution is more protective (4). It is possible that the presence of VPI salts solution and CO_2 in the gassed container may have been protective in this regard.

The value of the gassed transport system is less apparent when microorganisms are suspended in nutrient broth or when a mixed flora of aerobes and anaerobes is present. Both *B. fragilis* and *P. intermedius* survived almost equally well under aerobic conditions when suspended in broth. Others have noted similar oxygen tolerance of these anaerobes (5). However, aerobic organisms suspended in broth consistently increased in counts both in the gassed and the aerobic control tubes. This continued replication is undoubtedly due to growth-promoting nutrients present in the broth and is more pronounced for aerobes than for obligate anaerobes when both are present simultaneously. Thus, relative proportions of *B. fragilis* and *S. aureus* were appreciably altered in favor of the latter when both were mixed together in broth suspension but were relatively unchanged when suspended in saline.

These findings suggest that the major advantage of the gassed transport system may be for holding of specimens collected by saline irrigation, especially if mixed aerobic and anaerobic pathogens are suspected. Further studies using clinical specimens rather than stock cultures of clinical isolates are necessary to document the true value of the gassed transport system for primary isolation of both anaerobic and aerobic bacteria.

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