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Effect of Aerobic and Anaerobic Atmospheres on Isolation of Organisms from Blood Cultures

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Blood was cultured in two vacuum bottles containing Columbia broth. Filtered air was admitted to one bottle (aerobic); the unvented bottle was considered anaerobic. Cultures were incubated at 35 C until growth occurred or for at least 7 days. Of 744 organisms isolated, 50% were isolated from both bottles, 30% from the aerobic bottle only, and 20% from the anaerobic bottle only. These results indicate the need for use of both an aerobic and anaerobic bottle for blood cultures.

It is generally recommended that blood cultures be performed both aerobically and anaerobically. In practice this often consists of thioglycolate or thiol (Difco) broth for anaerobes and some type of nutrient broth, such as Trypticase soy or Columbia, for aerobes. Another common method is to use two vacuum bottles containing a suitable medium and to let filtered air into one, making it aerobic, with the unvented bottle then considered anaerobic. Because the use of two such culture bottles increases workload and requires more blood, at times only one bottle may be inoculated. In that circumstance it has been recommended that this bottle be kept anaerobic (2), since most facultative or "aerobic" organisms would be expected to grow anaerobically. The purpose of this study was to ascertain if both aerobic and anaerobic cultures are necessary for blood, or if, indeed, most organisms could be isolated in an anaerobic bottle alone.

MATERIALS AND METHODS

Blood was cultured and examined as previously described (1) over a period of 12 months. Two vacuum bottles containing 100 ml of Columbia broth with 0.03% sodium polyanethol sulfonate and 10% CO_2 (B-D Division of BioQuest or Hospital Service Technology Corp., North Andover, Mass.) were used for each blood culture. The bottles were inoculated at the bedside by physicians who were instructed to inoculate 10 ml of blood into each bottle; however, the amount of blood inoculated varied from a few drops to 10 ml. Upon receipt in the laboratory, filtered air was admitted to one bottle by using a blood collecting set; the collecting set was removed from the bottle before incubation. This bottle was considered to be aerobic, and the unvented bottle was considered anaerobic. Both bottles were incubated at 35 C.

Cultures were examined macroscopically for growth in the morning and afternoon of the day after they were received. Cultures that appeared positive were Gram stained, and subcultures were made according to the types of organisms seen.

All bottles which appeared negative by visualization were Gram stained after 1, 4, and 7 days of incubation. These bottles were also subcultured after 1 and 4 days of incubation; the aerobic bottle was inoculated onto chocolate agar (incubated in CO_2), and the anaerobic bottle was inoculated on a fresh sheep blood agar plate (incubated anaerobically). The subculture plates were incubated for 2 days before considering them negative.

All procedures were performed in the routine laboratory by a total of 13 microbiology technologists on a rotation basis.

When growth was initially detected (macroscopically, microscopically, or by subculture), it was noted whether it occurred in one or both of the bottles. If growth occurred in one bottle, the organism(s) was identified; the other bottle of the set was held at 35 C and examined for growth up to a maximum of 7 days (or 2 to 3 weeks in cases of suspected endocarditis or brucellosis). If growth occurred in one bottle on one day and in the other on any subsequent day, the culture was noted to have been detected in both bottles.

RESULTS

The organisms isolated and the bottle(s) in which they were detected are shown in Table 1.

Of 744 organisms, 375 (50%) were isolated in both bottles, 221 (30%) in the aerobic bottle only, and 148 (20%) in the anaerobic bottle only. The difference between the aerobic and anaerobic isolation rates was statistically significant ($P < 0.01$) using Cochran's χ^2 test.

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TABLE 1. *Number of organisms isolated from blood cultures aerobically, anaerobically, or both*

Organism	Total no. isolated	Aerobic and anaerobic		Aerobic only		Anaerobic only		$P(\chi^2)$[a]
		No.	%	No.	%	No.	%	
Acinetobacter	1	1	100	0	0	0	0	NS[b]
Bacillus	15	5	33	7	47	3	20	NS
Bacteroides fragilis	33	14	42	1	3	18	55	<0.01
Bacteroides ordlis	1	0	0	0	0	1	100	NS
Candida	28	6	21	22	79	0	0	<0.01
Citrobacter	2	1	50	0	0	1	50	NS
Clostridium	16	9	56	3	19	4	25	NS
Corynebacterium	28	4	14	15	54	9	32	NS
Cryptococcus neoformans	6	0	0	6	100	0	0	<0.05
Diplococcus pneumoniae	23	14	61	4	17	5	22	NS
Enterobacter	27	18	67	4	15	5	18	NS
Escherichia coli	96	67	70	16	17	13	13	NS
Fusobacterium	3	3	100	0	0	0	0	NS
Haemophilus influenzae	6	5	83	1	17	0	0	NS
Klebsiella	42	31	74	8	19	3	7	NS
Lactobacillus	1	1	100	0	0	0	0	NS
Neisseria sp.	1	0	0	1	100	0	0	NS
Peptococcus	4	1	25	0	0	3	75	NS
Peptostreptococcus	1	0	0	0	0	1	100	NS
Propionibacterium	40	9	23	5	13	26	64	<0.01
Proteus	9	5	56	2	22	2	22	NS
Pseudomonas	51	19	37	26	51	6	12	<0.01
Salmonella	10	6	60	3	30	1	10	NS
Serratia	6	3	50	3	50	0	0	NS
Staphylococcus aureus	99	75	76	14	14	10	10	NS
Staphylococcus epidermidis	120	30	25	63	53	27	22	<0.01
Streptococcus, Group D	19	9	48	5	26	5	26	NS
Streptococcus, viridans	39	30	77	6	15	3	8	NS
Streptococcus, beta	11	9	82	2	18	0	0	NS
Torulopsis glabrata	4	0	0	4	100	0	0	NS
Veillonella	2	0	0	0	0	2	100	NS

[a] Aerobic or anaerobic isolation only.
[b] NS, Not significant.

Among the anaerobes isolated, 14 of 33 (42%) strains of *Bacteroides fragilis* were isolated in the aerobic as well as the anaerobic bottle, one strain was isolated only in the aerobic bottle, and 18 (55%) strains were isolated in the anaerobic bottle only ($P < 0.01$). There were 16 strains of *Clostridium*, with nine (56%) isolated both aerobically and anaerobically, three isolated only aerobically, and four isolated only anaerobically. Those species isolated aerobically included *C. perfringens*, *C. tertium*, *C. septicum*, and *C. ramosum*. All three strains of *Fusobacterium* were isolated aerobically as well as anaerobically, and one of four strains of *Peptococcus* was also isolated from the aerobic bottle. Although the anaerobes cited above were isolated from the aerobic bottle, none of them except *C. tertium* grew on aerobic subculture plates.

Among the organisms considered to be aerobic, 26 (51%) of *Pseudomonas* were isolated only in the aerobic bottle and six (12%) were isolated only anaerobically ($P < 0.01$); nineteen (37%) were isolated from both bottles. One strain of *Neisseria* was isolated only from the aerobic bottle.

Among the facultative organisms there was a wide variability in the type of growth condition leading to isolation; most were isolated from both bottles, but many were isolated only from one bottle or the other (see Table 1). Of the 192 strains of *Enterobacteriaceae* isolated, 36 (19%) were isolated only from the aerobic bottle, and 25 (13%) were isolated only from the anaerobic bottle. Two hundred and nineteen staphylococci were isolated; of these, 77 (35%) were isolated only aerobically and 37 (17%) were isolated only anaerobically. These differences were significant only with the 120 strains of *Staphylococcus epidermidis* ($P < 0.01$). Out of

69 strains of streptococci, 13 (19%) were isolated aerobically, and 8 (12%) were isolated from the anaerobic bottle only.

The majority (79%) of *Candida* were isolated only aerobically ($P < 0.01$), and all of six strains of *Cryptococcus neoformans* ($P < 0.05$) and four strains of *Torulopsis glabrata* were isolated only aerobically.

DISCUSSION

The results of this study might appear paradoxical; anaerobes were isolated from an aerobic culture and aerobes were isolated from anaerobic cultures. There is no question that variability in the amount of blood inoculated into each bottle, and hence in the number of organisms inoculated, is responsible for some of these differences. It is also possible that in low levels of bacteremia organisms may not have been inoculated into both bottles. In addition, what is called an "aerobic" bottle evidently is not truly so. Letting some air in is unquestionably a help to organisms requiring oxygen (e.g., *Candida*, *Cryptococcus*) but true aerobiosis is not achieved, as evidenced by growth of anaerobic organisms which did not grow aerobically on subculture. There is a possibility that reducing substances in the blood and the growth of the organisms themselves tended to maintain a relatively low Eh in the bottle, and, due to the diffusion gradient, a relative anaerobic environment may be maintained near the bottom of the flask.

Other studies (3-5) of blood culture methodology have covered an assessment of various media for isolation of aerobes, facultatives, and anaerobes, with the emphasis primarily being on the efficacy of different types of media. There was no attempt made in those studies to systematically determine the effect of the incubation atmosphere on blood cultures, and none of the bottles were vented. However, the results of one investigation (5) did indicate a decreased isolation of aerobes and facultatives from anaerobic prereduced broth.

Although this study was carried out using only Columbia broth, the results do point out the advantages of using both "aerobic" and "anaerobic" bottles for blood cultures, both for more rapid detection of growth and for increased yield of organism isolation. In part, the difference in yield reflects the aliquoting of the sample into two culture bottles, but it is obvious from a review of the pattern of organism isolation that some organism groups fare poorly in one environment or the other. This is especially notable with those organisms having a known requirement for aerobic or anaerobic conditions, as shown by the significant results with *Bacteroides*, anaerobic cocci, *Pseudomonas*, and yeasts. The recommendation previously made (2) to use an anaerobic method if only one culture bottle was available would have resulted in missing 30% of the organisms in this study, whereas if only the aerobic bottle had been used 20% of the organisms would have been missed. The use of either environment alone would result in an unacceptably high error, and every effort should be made to inoculate both an aerobic and anaerobic blood culture bottle.

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