JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1982, p. 437–442
0095-1137/82/090437-06$02.00/0

Vol. 16, No. 3

Controlled Evaluation of the Effect of Atmosphere of Incubation on Detection of Bacteremia and Fungemia in Supplemented Peptone Broth

JAMES H. TENNEY,[1]† L. BARTH RELLER,[1,2]* STANLEY MIRRETT,[1,2] MELVIN P. WEINSTEIN,[1]‡ AND WEN-LAN L. WANG[3,4]

Clinical Microbiology Laboratory, University of Colorado Hospital,[2] Division of Infectious Diseases, Department of Medicine,[1] and Department of Pathology,[3] University of Colorado School of Medicine, Denver, Colorado 80262, and Microbiology Laboratory, Denver Veterans Administration Medical Center, Denver, Colorado 80220[4]

Received 25 January 1982/Accepted 27 May 1982

To evaluate the role of atmosphere of incubation in the detection of clinically important bacteremia and fungemia in adults, we compared the yield of microorganisms from 10,541 paired 5-ml samples of blood incubated aerobically and anaerobically. The medium, supplemented peptone broth (SPB) with 0.03% sodium polyanetholesulfonate, and the ratio of blood to broth (1:10) were the same for all cultures. Only cultures with adequate blood samples (≥80% of stated volume) were compared statistically. More fungi ($P < 10^{-7}$) grew in continuously vented bottles of SPB. Aerobic incubation also favored ($P < 0.01$) isolation of *Neisseria gonorrhoeae* and *Eubacterium*; more than 80% of these bacterial organisms were detected only in vented bottles. Anaerobic incubation (plugged venting units) did not significantly favor the isolation of any genus of microorganisms, although an estimated 11% more *Bacteroidaceae* grew in the unvented bottle of SPB. By comparison of our data with published results for other media, we conclude that the need for both aerobic and anaerobic incubation of blood cultures is dependent upon the medium used and the microorganisms likely to be encountered. Vented incubation of blood cultured in SPB is crucial for detection of fungi and some bacteria. Routine use of an unvented bottle of SPB may not be worthwhile for patients in whom *Bacteroidaceae* cause bacteremia infrequently. However, when *Bacteroidaceae* are suspected as the cause of sepsis, use of an unvented bottle of SPB is prudent.

With the advent of commercially prepared vacuum blood culture systems, most authorities have recommended use of both vented (aerobic) and unvented (anaerobic) bottles of broth media for optimal recovery of microorganisms from blood (1, 17). There are data to support the need for both aerobic and anaerobic incubation with Columbia broth and tryptic soy broth (TSB) (2, 7, 17). Whether vented and unvented bottles are important for all media used in commercial vacuum blood culture systems is not known. Therefore, we studied the role of atmosphere of incubation in the detection of bacteremia and fungemia with supplemented peptone broth (SPB) in a controlled clinical trial.

† Present address: Departments of Medicine and Pathology, University of Maryland Hospital, Baltimore, MD 21201.

‡ Present address: Division of Immunology and Infectious Diseases, Department of Medicine, College of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, NJ 08854.

MATERIALS AND METHODS

Collection of samples. For a 21-month period, two 50-ml bottles of SPB with 0.03% sodium polyanetholesulfonate (SPS; Becton Dickinson VACUTAINER Systems, Rutherford, N.J.) were used for all blood cultures from adult patients at the University of Colorado Hospital and Denver Veterans Administration Medical Center. House staff and medical students obtained the cultures at the bedsides of patients after preparation of the skin with a 10% povidone-iodine solution (1% available iodine), followed by 70% isopropyl alcohol. Blood from each separate venipuncture was distributed by needle and syringe as available (approximately 5 ml of blood to each of two bottles with 45 ml of SPB). Thus, for properly filled bottles, the volume of blood and the 1:10 ratio of blood to broth were the same for both culture bottles (1, 16, 17). The bottles were under vacuum and had been evacuated and back-flushed with 10% carbon dioxide in nitrogen at stoppering in the manufacturing process.

Processing of samples. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at both hospitals. When the paired

samples were received, a sterile, open venting unit that allowed continuous exchange of air was placed on one bottle, and a paraffin-petroleum jelly (Vaspar) plugged unit was placed on the other. Both bottles were incubated in a CO_2 incubator at 35°C. Subcultures of the aerobic (vented) bottle were done through the needle of the open venting unit, whereas those of the anaerobic bottle (plugged venting unit) were done with a separate needle and syringe. Blind anaerobic and aerobic subcultures were performed for both aerobic and anaerobic bottles according to the same schedules. Schedules and methods for macroscopic and microscopic examinations of bottles and subcultures and identification of microorganisms from positive cultures have been presented in detail previously (16).

Eubacterium organisms were defined as obligately anaerobic, nonbranching, nonbifid, nonsporeforming, gram-positive rods which failed to produce catalase and did not ferment lactose. Gas-liquid chromatography was used to support the identification of *Eubacterium*.

Analysis of data. Paired comparisons of aerobic and anaerobic bottles were done only on adequately filled (≥4 ml of blood) bottles that grew microorganisms causing true bacteremia or fungemia. Criteria for volume standards and clinical assessments have been described previously (16). Significance testing was done with the modified chi-square test described by McNemar (10). Standard errors for comparisons of media and atmospheres that favored isolation of particular microorganisms were derived from standard statistical formulas (9, 11).

RESULTS

A total of 12,179 blood culture sets were obtained during the study; 10,541 (87%) sets had both 50-ml bottles adequately filled, 1,093 (9%) sets had one or both bottles inadequately filled, and 545 (4%) sets had one bottle missing. Of the 10,541 adequate blood culture sets, 1,092 (10.4%) were positive, including 817 (7.8%) that grew microorganisms causing illness, 245 (2.3%) that grew contaminants, and 30 (0.3%) that grew at least one contaminant and one pathogen. A total of 955 microorganisms associated with sepsis were isolated from 10,541 adequately filled pairs of 50-ml bottles (Table 1). Of these 955 clinically important microorganisms, 602 (63.1%) grew in both the aerobic and anaerobic bottles.

Growth of fungi (specifically *Candida* and *Cryptococcus neoformans*) was strongly favored ($P < 10^{-7}$) by vented incubation (Table 1). Two genera of bacteria, *Neisseria* (specifically *Neisseria gonorrhoeae*) and *Eubacterium*, were isolated significantly more often ($P < 0.01$) from the vented bottle (Table 1). No genus of microorganisms was isolated significantly more frequently from the unvented bottle. There were no significant differences in speed of detection of microorganisms when the aerobic and anaerobic bottles were compared.

Eubacterium organisms were isolated from seven patients (four with polymicrobial bacteria) in 12 adequately filled culture sets (6 sets from one patient). *Eubacterium* would not have been isolated from five patients without vented incubation of their blood cultures. In two patients, *Eubacterium* sepsis was detected only by blind subculture of a vented bottle after 14 days of incubation.

Eubacterium grew from both bottles in 2 culture sets, but only in the continuously vented bottle in the other 10 sets (Table 1). In four culture sets, *Eubacterium* grew only in the vented bottle and was the only isolate from that bottle. In six additional culture sets, *Eubacterium* grew only in the vented bottle and was accompanied by facultative organisms only (four bottles; three *Escherichia coli*, one group F streptococcus) or by *Bacteroides* (two bottles). In these six culture sets, the paired unvented bottles grew facultative organisms only (five *E. coli*, one group F streptococcus). In the remaining two culture sets, *Eubacterium* grew in all four bottles and was accompanied by *Bacteroides* in each bottle. Blind anaerobic subcultures of the vented bottle proved to be the most rapid method of detection of *Eubacterium* in 8 of the 12 culture sets (1 on day 1 of incubation, 2 on day 7, and 5 on day 14). *Eubacterium* grew in association with other bacteria in 10 culture bottles (8 vented, 2 unvented), and blind subculture of an already positive bottle was required for detection of *Eubacterium* in 7 bottles (6 vented, 1 unvented).

DISCUSSION

We compared our results for the expected percentage of isolates that would be missed without aerobic or anaerobic incubation (Table 2, SPB) with the results from two other well-controlled clinical studies of the effect of atmosphere of incubation on detection of bacteremia and fungemia (2, 17). These studies used different media, Columbia broth with SPS or TSB with SPS; both studies used paired 10-ml samples of blood and vented vacuum bottles only transiently for aerobic incubation. We excluded *Bacillus, Corynebacterium, Propionibacterium*, and *Staphylococcus epidermidis* from the comparisons, since these organisms are most often contaminants.

When compared with unvented cultures, blood cultures that were vented to permit entry of air enabled significantly more isolations of microorganisms from the following seven genera in one or more of the three studies (Table 2, aerobic): *Candida* (three studies); *Cryptococcus* and *Pseudomonas* (two studies); and *Klebsiella, Acinetobacter, Neisseria*, and *Eubacterium* (one study each). Conversely, unvented incubation

TABLE 1. Comparison of yield of clinically important bacteria and fungi from 5-ml samples of blood incubated aerobically and anaerobically in SPB (50-ml bottles)

Microorganism	No. of isolates cultured from:			P
	Both bottles	Vented bottle only	Unvented bottle only	
Aerobic and facultative bacteria	534	141	113	NS[a]
Gram-positive	305	61	47	NS
Staphylococci	138	26	19	NS
Streptococci	156	27	22	NS
Other[b]	11	8	6	NS
Gram-negative	229	80	66	NS
E. coli	100	26	30	NS
Klebsiella	50	10	10	NS
Other *Enterobacteriaceae*[c]	36	14	14	NS
Pseudomonas	36	16	9	NS
N. gonorrheae	2	11	0	<0.01
Other[d]	5	3	3	NS
Anaerobic bacteria	51	29	24	NS
Eubacterium	2	10	0	<0.01
Bacteroidaceae	34	10	17	NS
Other[e]	15	9	7	NS
All bacteria	585	170	137	NS
Fungi	17	42	4	$<10^{-7}$
Candida	10	31	2	$<10^{-6}$
C. neoformans	1	8	0	<0.02
Other[f]	6	3	2	NS

[a] NS, Not significant ($P > 0.05$).
[b] *Listeria* (13), *Corynebacterium* (10), *Lactobacillus* (1), and *Bacillus* (1).
[c] *Serratia* (20), *Proteus* (17), *Enterobacter* (14), *Providencia* (5), *Morganella* (3), *Salmonella* (3), and *Citrobacter* (2).
[d] *Haemophilus influenzae* (5), *Acinetobacter* (3), *Pasteurella* (1), *Eikenella* (1), and an unidentified gram-negative rod (1).
[e] *Clostridium* (12), *Peptococcus* (10), *Peptostreptococcus* (8), and *Veillonella* (1).
[f] *Torulopsis glabrata* (10) and *Mucor* (1).

significantly favored the isolation of microorganisms from the following four organism groups (Table 2, anaerobic): *Bacteroidaceae* (two studies) and *Escherichia, Haemophilus*, and *Peptococci* (one study each). Three-way comparison of the effect of media on the importance of vented or unvented incubation was possible for five of these organism groups, *Candida, Pseudomonas, Klebsiella, Bacteroidaceae*, and *Escherichia*. For detection of *Pseudomonas* (Table 2), the estimated advantage of aerobic incubation was much greater for Columbia broth (≈40%) than for either TSB or SPB (10 to 20%). For detection of *Bacteroidaceae* (Table 2 and Fig. 1), the estimated advantage of anaerobic incubation was much greater for Columbia broth (≈50%) than for either TSB or SPB (10 to 15%). The estimated advantage of aerobic or anaerobic incubation for isolation of *Candida, Klebsiella*, and *Escherichia* did not differ significantly among media.

Investigators examining conditions required for isolation of fungi have repeatedly stressed the need for aerobic incubation for both yeasts and filamentous fungi (2, 3, 6, 12, 17). Our data for *Candida* and *Cryptococcus* confirm the requirement for vented incubation in SPB. In our study, an estimated 60% (38 of 63) of fungi would not have been isolated without a vented culture bottle.

The importance of an aerobic atmosphere for optimum growth of *Pseudomonas* was demonstrated in early studies by Slotnick and Sachs (14) and Knepper and Anthony (8). Braunstein and Tomasulo (4) suggested experimentally that blind aerobic subculture of unvented bottles

TABLE 2. Genera of microorganisms causing septicemia whose detection in three clinical blood culture trials was favored by aerobic or anaerobic incubation

Microorganisms favored by aerobic incubation	Expected % (±2 SE) of isolates missed without aerobic incubation[a]		
	Columbia broth[b]	Tryptic soy broth[c]	Supplemented peptone broth[d]
Candida	**79** (±16)	**76** (±11)	**67** (±17)
Crytococcus	**100**[e]	—	**89**[e]
Pseudomonas	**39** (±19)	**19** (±7)	11 (±16)
Klebsiella	12 (±16)	**10** (±7)	0 (±13)
Acinetobacter	—	**35** (±26)	—
Neisseria	—	—	**85**[e]
Eubacterium	—	—	**83**[e]

Microorganisms favored by anaerobic incubation	Expected % (±2 SE) of isolates missed without anaerobic incubation[a]		
	Columbia broth[b]	Tryptic soy broth[c]	Supplemented peptone broth[d]
Bacteroidaceae	**49** (±18)	**14** (±8)	11 (±17)
Escherichia	−3[f] (±11)	**5** (±4)	3 (±10)
Haemophilus	—	**24** (±16)	—
Peptococcus	—	**88**[e]	—

[a] The percentage is reported as $100(|x - y|)/n$, and the two standard errors are reported as

$$\left(\pm 200/n\sqrt{x[1 - x/n] + y[1 - y/n] + 2xy/n}\right),$$

where x is the number of isolates detected only by aerobic incubation, y is the number of isolates detected only by anaerobic incubation, and n is the total number of isolates from each genus. Boldface numbers indicate percentages significantly ($P < 0.05$) different from 0%. —, Insufficient isolates to permit a valid statistical analysis.

[b] See reference 2; total isolates reported, 541; *Bacillus, Corynebacterium, Propionibacterium*, and *Staphylococcus epidermidis* are omitted.

[c] See reference 17; total isolates reported, 3,505; *Bacillus, Corynebacterium, Propionibacterium*, and *Staphylococcus epidermidis* are omitted.

[d] Present report.

[e] Insufficient isolates to permit calculation of standard error.

[f] Three percent more *E. coli* were isolated in the vented bottle than in the unvented bottle.

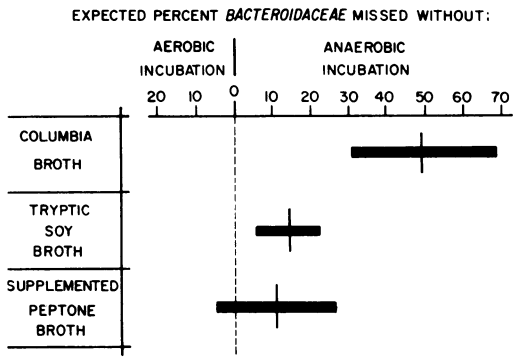

FIG. 1. Importance of anaerobic incubation for the detection of *Bacteroidaceae* in blood cultures: comparison of three controlled clinical studies, using Columbia broth (Blazevic et al. [2]), TSB (Washington [17]), and SPB (this report). Data from Table 2 are represented as expected percent *Bacteroidaceae* missed without anaerobic incubation (±2 standard errors).

should enable isolation of *Pseudomonas*; however, controlled clinical trials with appropriate subculturing have demonstrated that introduction of air into blood culture bottles containing an anaerobic atmosphere is still necessary for optimum yield of *Pseudomonas* in Columbia broth and TSB (2, 17). Our data (Table 2) are consistent with the conclusion that vented incubation favors the isolation of *Pseudomonas* in SPB as well.

Of 13 isolates of *N. gonorrhoeae* in this study, 11 grew only in the vented bottle of SPB. Too few *Neisseria* organisms were recovered in the other two studies to enable comparisons (2, 17). SPB with 0.03% SPS contains 1.2% gelatin, which neutralizes the inhibitory effect of SPS on *N. gonorrhoeae, Neisseria meningitidis*, and *Peptostreptococcus anaerobius* (5, 15, 18). Neither TSB nor Columbia broth used in the other two studies contained gelatin.

Both vented incubation and careful blind subculturing were necessary to provide optimal detection of *Eubacterium* in SPB. We were surprised that vented incubation of SPB was crucial for isolation of *Eubacterium*, an obligate anaerobe. This finding could not be explained by multiple isolations of a single strain from one patient, since aerobic incubation of blood cultures was required for detection of *Eubacterium* sepsis in five of seven patients. The data suggest that vented incubation of SPB facilitates the isolation of *Eubacterium* when it is present alone and when it is the only anaerobe in the company of more rapidly growing facultative organisms. Too few *Eubacterium* organisms were isolated from the studies with Columbia broth and TSB to evaluate the importance of aerobic incubation for detection of *Eubacterium* in these media. Our data suggest that blind subcultures at 14 days and repeated subcultures of previously positive bottles are needed to provide optimal detection of sepsis with *Eubacterium*. These procedures were not performed during the studies of Blazevic et al. (2) and Washington (17).

We and others have concluded that at least 10 ml of blood should be sampled for each culture (16, 17). How should we evaluate whether to

divide this volume of blood and use both aerobic (vented) and anaerobic (unvented) incubation with SPB or other media? A judgment in favor of a two-bottle system cannot be based simply on detection of organism groups, some significantly ($P < 0.05$) favored by vented incubation and others significantly favored by unvented incubation. Such a judgment is highly dependent on the length of the clinical trial performed. For example, the early trial by Harkness et al. (7) found that atmosphere of incubation made a significant difference for only two organism groups, *Candida* and *Pseudomonas*, both favored by vented incubation. The extension of that same study (17), however, showed that four organism groups were significantly favored by vented and four by unvented incubation (Table 2, TSB). This paradox can be resolved by comparing the expected detection of isolates from important organism groups for three methods of culturing the blood sample: (i) the entire sample incubated aerobically; (ii) the entire sample incubated anaerobically; and (iii) one-half of the sample incubated aerobically and the other half incubated anaerobically (two-bottle system).

A two-bottle system for culturing blood to permit vented (aerobic) and unvented (anaerobic) incubation requires almost double the expense in technician time, culture material, and laboratory space compared with a one-bottle system. This substantial additional cost must be measured against the benefit of detecting additional microorganisms. For each organism group, the benefit may be assessed by the following four factors: (i) the clinical importance of the microorganism, (ii) the incidence of septicemia with that organism for the particular clinical setting (e.g., hospital, service, or patient group), (iii) the expected percentage of isolates missed by omitting vented or unvented incubation (Table 2 and Fig. 1), and (iv) the precision of that estimate (Table 2 and Fig. 1).

For each medium, Columbia broth, TSB, or SPB, a substantial percentage of clinically important, frequently isolated organisms would be missed without vented incubation (Table 2). Thus, vented incubation is indicated for all three media. The advantages of unvented incubation are less pronounced, and analysis may appropriately be focused on the *Bacteroidaceae* (Table 2 and Fig. 1), clinically important microorganisms for which unvented incubation may be advantageous. About 50% of *Bacteroidaceae* required unvented incubation for detection in Columbia broth. Thus, unvented incubation and, therefore, a two-bottle system would be important for Columbia broth in most clinical settings. In SPB, however, only an estimated 11% of *Bacteroidaceae* required unvented incubation. It seems sensible to use vented incubation of SPB for the entire volume of blood sampled from patients with hematological malignancies in whom *Bacteroidaceae* sepsis is infrequent and *Pseudomonas* sepsis is much more common (13), because the very small number of *Bacteroidaceae* organisms missed without anaerobic incubation would be outweighed by the number of additional *Pseudomonas* organisms detected by the additional volume of blood available for aerobic incubation (16, 17). Conversely, however, if *Bacteroidaceae* sepsis is suspected, the use of an unvented bottle with SPB is prudent. Figure 1 shows graphically that judgments with TSB should parallel those with SPB.

These empirical judgments about the need for both aerobic and anaerobic incubation depend upon the specific culture systems used in the clinical trials analyzed. Information on the difference in redox potentials (Eh) between vented and unvented conditions is unavailable for commercially produced blood culture media. Presumably, it is this medium-specific difference that results in different isolation rates for microorganisms. Use of both aerobic and anaerobic incubation implies the need for incubation of blood cultures at two different Eh. What Eh to choose, and how far apart they should be, are questions for further study. Our data indicate that facile conclusions about the type of organism likely to be isolated at a particular Eh (e.g., *Eubacterium* at a lower Eh) and about the need for all media to be incubated aerobically and anaerobically in every clinical setting are unwarranted.

ACKNOWLEDGMENTS

We are grateful to J. Richard Hebel for expert statistical consultation, to David Vlahov for careful review of the manuscript, and to Freda Mason for manuscript preparation.

This work was supported in part by a grant from Becton Dickinson VACUTAINER Systems, Rutherford, N.J.

LITERATURE CITED

1. **Bartlett, R. C., P. D. Ellner, and J. A. Washington II.** 1974. Cumitech 1, blood cultures. Coordinating ed., J. C. Sherris. American Society for Microbiology, Washington, D.C.
2. **Blazevic, D. J., J. E. Stemper, and J. M. Matsen.** 1975. Effect of aerobic and anaerobic atmospheres on isolation of organisms from blood cultures. J. Clin. Microbiol. 1:154–156.
3. **Braunstein, H., and M. Tomasulo.** 1976. A quantitative study of the growth of *Candida albicans* in vented and unvented culture bottles. Am. J. Clin. Pathol. 66:87–90.
4. **Braunstein, H., and M. Tomasulo.** 1976. A quantitative study of the multiplication of *Pseudomonas aeruginosa* in vented and unvented blood-culture bottles. Am. J. Clin. Pathol. 66:80–86.
5. **Eng, J., and E. Holten.** 1977. Gelatin neutralization of the inhibitory effect of sodium polyanethol sulfonate on *Neisseria meningitidis* in blood culture media. J. Clin. Microbiol. 6:1–3.
6. **Gantz, N. M., J. L. Swain, A. A. Medeiros, and T. F. O'Brien.** 1974. Vacuum blood-culture bottles inhibiting

growth of *Candida* and fostering growth of *Bacteroides*. Lancet **ii**:1174–1176.
7. **Harkness, J. L., M. Hall, D. M. Ilstrup, and J. A. Washington II.** 1975. Effects of atmosphere of incubation and of routine subcultures on detection of bacteremia in vacuum blood culture bottles. J. Clin. Microbiol. **2**:296–299.
8. **Knepper, J. G., and B. F. Anthony.** 1973. Diminished growth of *Pseudomonas aeruginosa* in unvented blood-culture bottles. Lancet **ii**:285–287.
9. **Lindgren, B. W., and G. W. McElrath.** 1959. Introduction to probability and statistics, p. 93–94. MacMillan Publishing Co., New York.
10. **McNemar, Q.** 1962. Psychological statistics, 3rd ed., p. 209–239. John Wiley and Sons, New York.
11. **Rao, C. R.** 1962. Advanced statistical methods in biometric research, p. 34–35. J. Wiley & Sons, Inc., New York.
12. **Roberts, G. D., C. Horstmeier, M. Hall, and J. A. Washington II.** 1975. Recovery of yeast from vented blood culture bottles. J. Clin. Microbiol. **2**:18–20.
13. **Singer, C., M. H. Kaplan, and D. A. Armstrong.** 1977. Bacteremia and fungemia complicating neoplastic disease: a study of 364 cases. Am. J. Med. **62**:731–742.
14. **Slotnick, I. J., and H. J. Sachs.** 1972. The growth of *Pseudomonas* in blood cultures. Am. J. Clin. Pathol. **58**:723–725.
15. **Staneck, J. L., and S. Vincent.** 1981. Inhibition of *Neisseria gonorrhoeae* by sodium polyanetholsulfonate. J. Clin. Microbiol. **13**:463–467.
16. **Tenney, J. H., L. B. Reller, S. Mirrett, W.-L. L. Wang, and M. P. Weinstein.** 1982. Controlled evaluation of the volume of blood cultured in detection of bacteremia and fungemia. J. Clin Microbiol. **15**:558–561.
17. **Washington, J. A., II.** 1978. Conventional approaches to blood culture, p. 69–73. *In* J. A. Washington II (ed.), The detection of septicemia. CRC Press, West Palm Beach, Fla.
18. **Wilkins, T. D., and S. E. H. West.** 1976. Medium dependent inhibition of *Peptostreptococcus anaerobius* by sodium polyanetholsulfonate in blood culture media. J. Clin. Microbiol. **3**:394–396.