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Critical Assessment of Blood Culture Techniques: Analysis of Recovery of Obligate and Facultative Anaerobes, Strict Aerobic Bacteria, and Fungi in Aerobic and Anaerobic Blood Culture Bottles

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Recent reports have documented a decrease in anaerobic bacteremias and have questioned the need for routine anaerobic blood cultures. At the same time, we and others have noted an increase in fungal bloodstream infections. In this two-part study, we first compared recoveries of obligate anaerobic bacteria with those of fungi over a 13-year period and then examined the recoveries of all bacteria and fungi in aerobic and anaerobic blood culture bottles during a 12-month period. During the 13-year period, the number of patients with anaerobic bacteremia remained relatively constant (average, 39 patients per year), while the incidence of fungemia steadily increased, from 12 patients in 1978 to 117 patients in 1990. Of the 1,090 anaerobic isolates, 55.1 and 90.2% were recovered in aerobic and anaerobic bottles, respectively, compared with 98.6 and 37.0% of the 2,582 fungi. During the 12-month period of evaluation, 2,980 bacteria and fungi were recovered in cultures collected from 1,555 patients. Overall, 21.1% more organisms were recovered in aerobic bottles than in anaerobic bottles, including significantly more *Staphylococcus* species; gram-positive aerobic bacilli; *Escherichia*, *Enterobacter*, *Pseudomonas*, *Xanthomonas*, and *Acinetobacter* species; miscellaneous gram-negative bacilli; and yeasts. Only anaerobic gram-negative bacilli and non-spore-forming gram-positive bacilli were isolated more commonly in anaerobic bottles. These data support the concepts that bacteremia caused by obligate anaerobic bacteria is decreasing relative to sepsis caused by other bacteria and fungi and that the routine use of unvented anaerobic blood culture bottles reduces the recovery of common aerobic bloodstream pathogens.

The current recommendations for culturing blood collected from patients with presumed sepsis include division of the specimens equally between two types of culture bottles for the recovery of aerobic and anaerobic bacteria and the selective use of a fungal blood culture system when fungemia is suspected clinically (1, 30, 32, 37). This approach is logical when viewed in a historical perspective. Studies comparing the recoveries of organisms in blood culture bottles demonstrated that anaerobes grew preferentially in unvented (anaerobic) bottles, whereas strict aerobic organisms such as yeasts and *Pseudomonas* and *Neisseria* species grew preferentially in vented (aerobic) bottles (2, 8, 9, 33, 36). Wilson and associates (41) reported in 1972 that strict anaerobes were isolated in 26.3% of the positive blood cultures collected at the Mayo Clinic, and other investigators reported that between 10 and 20% of their blood cultures yielded anaerobes (1, 24, 26). The selective use of fungal culture systems has been recommended when fungemia is suspected clinically because early studies demonstrated that fungi grew best in aerated or biphasic culture bottles (28, 33), while more recent studies have demonstrated enhanced recovery of fungi in the lysis-centrifugation system (3, 11) or agitated biphasic bottles (22).

Recent reports have indicated, however, a change in the distribution of organisms recovered in blood cultures. The decreased incidence of anaerobic bacteremia has been documented by Dorsher et al. (4) and reviewed by Sharp (34). In contrast, fungal infections have increased dramatically in recent years, particularly in immunocompromised patients and patients previously treated with broad-spectrum antibacterial agents (6, 12, 16, 17, 19, 27, 39, 40). Because the success of detecting bacteria and fungi in blood cultures is directly related to the volume of blood that is cultured (7, 14, 18, 31, 35, 37), it seems logical to reconsider current blood culture practices. After reviewing the recent experience with anaerobic bacteremia, Sharp recommended the routine use of two aerobic blood culture bottles for optimum recovery of bacteria and fungi in blood and the selective use of anaerobic cultures (34).

What has been ignored in these considerations, however, is the possibility that some facultative anaerobic bacteria (e.g., staphylococci, streptococci, and members of the family *Enterobacteriaceae*) may grow better in the unvented anaerobic blood culture bottles. Whereas anaerobes may be uncommon blood culture isolates, the majority of bacteria responsible for sepsis are facultative anaerobes. Thus, the major benefit of the unvented blood culture broths may be for the recovery of facultative anaerobic bacteria that preferentially grow in anaerobic bottles.

The purpose of the study reported herein was twofold. First, the incidence of anaerobic bacteremia over a 13-year period was examined and compared with the incidence of fungemia over the same period of time. This portion of the study was designed to determine the relative values of the aerobic and anaerobic culture systems for the isolation of these two specific groups of organisms. The second part of the study examined the frequency with which all blood

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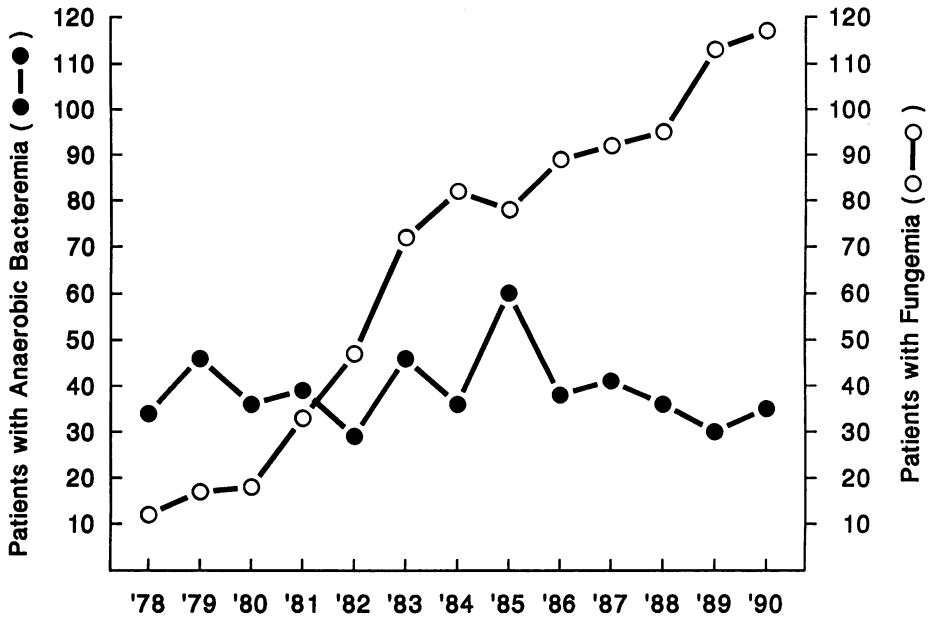

FIG. 1. Annual number of patients with bacteremia caused by obligate anaerobic bacteria and with fungemia from 1978 to 1990.

culture organisms isolated during a 1-year period grew in either the aerobic or the anaerobic blood culture bottles.

MATERIALS AND METHODS

Study period. The laboratory records of recoveries of all obligate anaerobes and fungi from 1978 to 1990 were reviewed. Additionally, the laboratory records of all blood culture isolates for a 1-year period from May 1990 to April 1991 were reviewed.

Blood culture systems. During the 13-year study period, a number of changes were incorporated in the methods used for processing blood cultures. The following is a summary of these changes. From 1978 through 1981, each blood specimen was inoculated into two types of 100-ml blood culture bottles: a transiently vented tryptic soy broth bottle (Difco Laboratories, Detroit, Mich.) and an unvented Thiol broth bottle (Difco). The bottles were examined daily for macroscopic evidence of growth, and the vented bottles were subcultured onto chocolate blood agar on days 1 and 3 and incubated at 35°C for 48 h (29). All blood culture bottles were incubated for a total of 14 days. In 1982, the tryptic soy broth bottles were replaced with the Roche biphasic tryptic soy broth bottles with the Septi-Chek agar slide unit (Roche Diagnostics [division of Hoffmann-La Roche, Nutley, N.J.]). The biphasic bottles were incubated for a total of 14 days and processed as previously described (28). In 1984, the unvented Thiol broth bottles were replaced with unvented Columbia broth bottles (Roche Diagnostics). Incubation of these bottles was decreased from 14 to 7 days because no significant isolates were detected in the unvented bottles after the first week of incubation (21). The biphasic bottles were incubated for 14 days during the entire study period. Beginning in 1986, an additional 10 ml of blood was collected in an Isolator 10 vacuum tube (Wampole Laboratories, Cranbury, N.J.) and processed as previously described for patients with suspected fungemia (22). In 1988, we initiated agitation of the aerobic biphasic blood culture bottles on a mechanical mixer (150 rpm; New Brunswick Scientific Co., Inc., Edison, N.J.) for the first 24 h of incubation (23). In 1991, we replaced the Isolator culture with a second agitated biphasic blood culture bottle whenever fungemia was suspected (22, 25).

Identification of isolates. All aerobic and anaerobic bacteria as well as fungi were identified by standard methodologies (1). Single isolates of coagulase-negative staphylococci and *Bacillus*, *Corynebacterium*, and *Propionibacterium* species were considered to be contaminants and were excluded from further analysis.

Statistical analysis. The asymptotic chi-square test of McNemar as described by Ilstrup (13) was used to compare the recoveries of organisms in the aerobic and anaerobic bottles.

RESULTS

Recovery of anaerobic bacteria in blood cultures from 1978 to 1990 was compared with recovery of fungi during the same period (Fig. 1). The number of patients with anaerobic bacteremia remained relatively constant during the study period (average, 39 per year; range, 29 to 60), whereas the number of patients with fungemia steadily increased from 12 patients in 1978 to 117 patients in 1990. The number of blood cultures processed during the 13-year period (excluding specific fungal Isolator cultures) increased 278% from 11,862 cultures in 1978 to 33,001 cultures in 1990. When the number of blood cultures with anaerobic bacteria was examined, the proportion of positive cultures decreased from 6.2 per 1,000 cultures in 1978 to 1.6 per 1,000 processed cultures in 1990 (Fig. 2). In contrast, positive fungal cultures increased from 1.8 per 1,000 cultures in 1978 to 13.7 per 1,000 cultures in 1990.

During this 13-year period, the number of patients admitted annually into Barnes Hospital decreased from 40,554 to 32,860, and the annual number of patient-days of hospitalization (influenced by both admissions and lengths of stay) decreased from 367,579 to 266,652 days. The numbers of patients with anaerobic bacteremia and with fungemia per 1,000 hospital admissions and per 100,000 patient-days were

FIG. 2. Proportion of patients with anaerobic bacteremia or fungemia per 1,000 processed blood cultures.

also calculated (Fig. 3 and 4, respectively). In both analyses, the incidence of anaerobic bacteremia remained relatively constant, whereas the incidence of fungemia continued to increase. On the basis of these data, it appears that the importance of detecting anaerobic bacteremia has decreased relative to that of detecting fungemia.

The method by which the positive cultures were detected was then examined. The anaerobic bacteria that were isolated during the 13-year period, as well as the numbers of positive cultures in the bottles, are summarized in Table 1. It is not surprising that members of the *Bacteroides fragilis* group (isolated from 308 patients), in particular *B. fragilis* (214 patients), were the most commonly isolated anaerobes, followed by *Clostridium* species (isolated from 72 patients), *Fusobacterium* species (isolated from 50 patients), and *Peptostreptococcus* species (isolated from 35 patients). A total of 1,090 anaerobes were isolated, including 107 isolates in aerobic bottles only, 489 isolates in anaerobic bottles only, and 494 in both types of bottles. Of the 1,090 anaerobic isolates, 601 (55.1%) and 983 (90.2%) were recovered in aerobic and anaerobic bottles, respectively. Although better recovery of anaerobic bacteria was observed with unvented (anaerobic) bottles, as was predicted, more than one-half of the isolates were detected in aerobic bottles.

The method of recovery of fungi during the 13-year study period was also examined (Table 2). Fungemia in 867 patients was documented, with *Candida albicans* (from 378 patients), *Candida tropicalis* (from 193 patients), and *Toru-*

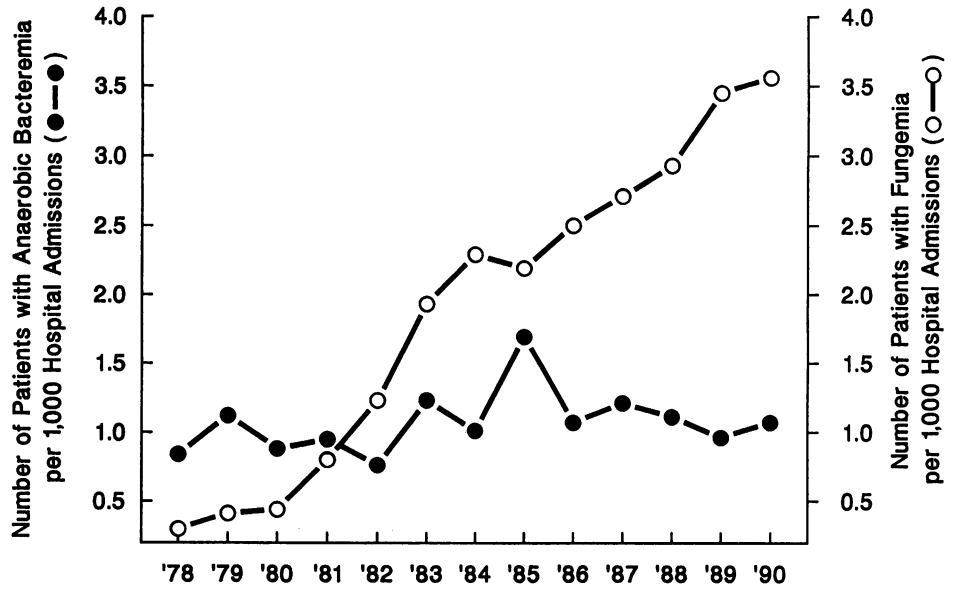

FIG. 3. Proportion of patients with anaerobic bacteremia or fungemia per 1,000 hospital admissions.

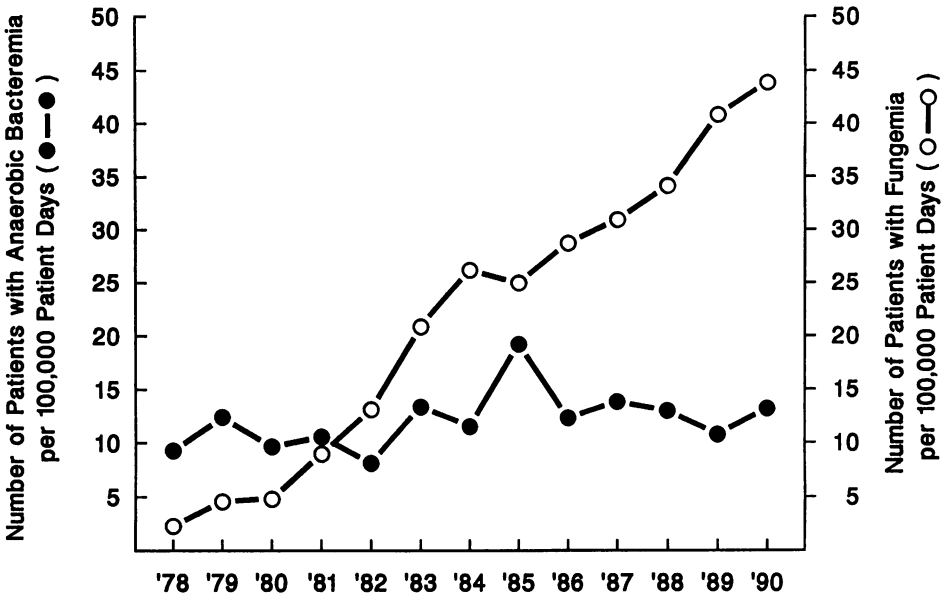

FIG. 4. Proportion of patients with anaerobic bacteremia or fungemia per 100,000 patient-days of hospitalization.

lopsis glabrata (from 138 patients) being the most common isolates. A total of 2,582 fungi were isolated, with positive cultures observed with 1,628 aerobic bottles only, 37 anaerobic bottles only, and 917 bottles of both types. Of the 2,582 fungal isolates, 2,545 (98.6%) and 954 (37.0%) were recovered in the aerobic and anaerobic bottles, respectively. Clearly, fungal growth was severely inhibited in anaerobic bottles.

TABLE 1. Anaerobic isolates at Barnes Hospital from 1978 to 1990

Anaerobe (no. of patients)	No. of positive cultures in:		
	Aerobic bottles only	Anaerobic bottles only	Both types of bottles
B. fragilis group			
B. fragilis (214)	40	184	220
B. thetaiotaomicron (48)	3	61	31
B. distasonis (17)	5	18	6
B. ovatus (17)	1	24	20
Other species (12)		10	7
Bacteroides species (28)	3	21	12
Fusobacterium species			
F. nucleatum (25)	6	17	15
Other species (25)	5	21	13
Prevotella species			
P. melaninogenicus (16)	3	13	8
P. bivia (11)	3	9	9
Other species (13)	3	8	4
Porphyromonas species (2)		4	
Clostridium species			
C. perfringens (34)	13	14	54
C. septicum (13)	1	5	19
Other species (25)	9	32	29
Eubacterium species (6)		7	5
Bifidobacterium species (3)	3	2	1
Lactobacillus species (2)	1	1	2
Actinomyces species (1)	1	2	
Peptostreptococcus species (35)	6	36	38
Veillonella species (1)	1		1

The overall recovery of all bacteria and fungi in aerobic and anaerobic bottles was then determined for a 12-month period from May 1990 to April 1991 (Table 3). A total of 2,980 bacteria and fungi were recovered in cultures of isolates collected from 1,555 patients, excluding single isolates of contaminants (refer to Materials and Methods). The most commonly isolated organisms were coagulase-negative staphylococci (from 285 patients) and *Staphylococcus aureus* (from 173 patients), viridans group *Streptococcus* species (from 122 patients), *Enterococcus* species (from 120 patients), *Escherichia* and *Klebsiella* species (from 156 and 95 patients, respectively), *Pseudomonas* species (from 94 patients), and *Candida* species (from 85 patients). A total of 121 (7.8%) of the 1,555 patients had fungemia, compared with 82 (5.3%) patients with anaerobic bacteremia.

A total of 874 isolates were present only in aerobic bottles, 429 were present only in anaerobic bottles, and 1,678 were present in both types of bottles. That is, 21.1% more organisms were recovered in the biphasic aerobic bottles than in the unvented anaerobic bottles. Organisms that were

TABLE 2. Fungal isolates at Barnes Hospital from 1978 to 1990

Fungus (no. of patients)	No. of positive cultures in:		
	Aerobic bottles only	Anaerobic bottles only	Both types of bottles
Candida albicans (378)	715	15	379
Candida tropicalis (193)	369	4	294
Candida parapsilosis (75)	129	5	57
Candida krusei (22)	48	2	31
Candida lusitaniae (6)	6		3
Candida pseudotropicalis (3)	9		1
Candida guilliermondii (2)	6		
Candida rugosa (1)	1		
Torulopsis glabrata (138)	196	10	131
Cryptococcus neoformans (42)	129		18
Trichosporon beigelii (2)	4	1	3
Histoplasma capsulatum (5)	16		

TABLE 3. Bacterial and fungal isolates from blood cultures collected from May 1990 to April 1991

Organism (no. of patients)	No. of positive cultures in:		
	Aerobic bottles only	Anaerobic bottles only	Both types of bottles
Staphylococcus aureus (173)	73	27	239
Staphylococcus species, coagulase negative (285)	289	133	320
Streptococcus species			
Viridans group (122)	46	37	99
Beta-hemolytic (50)	13	11	57
S. pneumoniae (45)	11	6	64
Enterococcus species (120)	38	33	87
Other gram-positive aerobes (22)[a]	21	2	20
Enterobacteriaceae			
Escherichia species (156)	58	32	190
Klebsiella species (95)	27	27	129
Enterobacter species (63)	22	8	98
Proteus species (32)	13	10	29
Other members of the family *Enterobactericeae* (31)	5	7	41
Pseudomonas species (94)	50	15	122
Xanthomonas species (19)	7	0	36
Acinetobacter species (24)	10	2	14
Other gram-negative aerobes (21)[b]	12	1	15
Bacteroides species (26)	3	27	2
Other gram-negative anaerobes (5)[c]	0	6	2
Peptostreptococcus species (4)	2	2	0
Clostridium species (21)	5	13	9
Other gram-positive anaerobes (26)[d]	8	20	7
Candida species			
C. albicans (52)	92	4	36
C. tropicalis (25)	29	4	17
Other *Candida* species (8)	7	0	7
Torulopsis species (25)	26	2	34
Cryptococcus species (10)	0	0	10

[a] *Corynebacterium* (13 patients), *Listeria* (5), *Aeroboccus* (2), *Bacillus* (1), and *Leukonostoc* (1) species.
[b] *Aeromonas* (4 patients), *Haemophilus* (4), *Moraxella* (3), *Neisseria* (3), *Flavobacterium* (2), *Alcaligenes* (1), *Campylobacter* (1), *Legionella* (1), *Pasteurella* (1), and *Actinobacillus* (1) species.
[c] *Veillonella* (3 patients), *Fusobacterium* (1), and *Porphyromonas* (1) species.
[d] *Propionibacterium* (10 patients), *Actinomyces* (7), *Lactobacillus* (6), and *Bifidobacterium* (3) species.

TABLE 4. Detection of bacteremia and fungemia in aerobic versus anaerobic blood culture bottles

Organism (no.[a])	No. of patients with positive cultures in:		
	Aerobic bottles only	Anaerobic bottles only	Both types of bottles
Staphylococcus aureus (97)	9	1	87
Staphylococcus species, coagulase negative (285)	44	14	227
Streptococcus species			
Viridans group (41)		3	38
Beta-hemolytic (22)	1	1	20
S. pneumoniae (36)	2	1	34
Enterococcus species (33)	2	1	30
Other gram-positive aerobes (15)[b]	7		8
Enterobacteriaceae (209)	16	9	184
Pseudomonas species (41)	3		38
Xanthomonas species (10)			10
Acinetobacter species (2)	1		1
Other gram-negative aerobic bacteria (7)[c]	1		6
Bacteroides species (3)		3	
Other gram-negative anaerobes (2)[d]		2	
Peptostreptococcus species (1)		1	
Clostridium species (5)		2	3
Other gram-positive anaerobes (11)[e]	4	3	4
Candida species (43)	18		25
Torulopsis species (15)	3		12
Cryptococcus species (7)	3		4

[a] Number of patients with two or more positive cultures.
[b] *Corynebacterium* (13 patients), *Listeria* (1), and *Bacillus* (1) species.
[c] *Actinobacillus* (1 patient), *Alcaligenes* (1), *Flavobacterium* (1), *Haemophilus* (1), *Legionella* (1), *Moraxella* (1), and *Neisseria* (1) species.
[d] *Fusobacterium* (1 patient) and *Porphyromonas* (1) species.
[e] *Propionibacterium* (10 patients) and *Lactobacillus* (1) species.

recovered significantly more often in aerobic bottles were *Staphylococcus aureus* and coagulase-negative *Staphylococcus* species ($P < 0.001$), gram-positive bacilli ($P < 0.001$), *Escherichia* species ($P < 0.01$), *Enterobacter* species ($P < 0.05$), *Pseudomonas* and *Xanthomonas* species ($P < 0.001$), *Acinetobacter* species ($P < 0.05$), miscellaneous gram-negative bacilli ($P < 0.01$), and yeasts ($P < 0.001$). The only organisms recovered significantly more often in anaerobic bottles were *Bacteroides* species and other gram-negative anaerobes and the anaerobic non-spore-forming gram-positive bacilli ($P < 0.05$).

The relative values of aerobic and anaerobic bottles for individual patients were examined. If the patient had small numbers of organisms intermittently present in the bloodstream, then microbial growth may be recovered in only one culture and one bottle. In this situation the isolation of an organism in one bottle may be due to chance or to the preference for one culture bottle. For this reason, recovery of organisms from patients with at least two positive cultures was analyzed (Table 4). A total of 885 patients had two or more positive cultures. Growth was detected only in aerobic bottles for 114 (12.9%) patients, only in anaerobic bottles for 40 (4.5%) patients (including 11 patients with obligate anaerobes and 29 patients with facultative anaerobes), and in both types of bottles for 731 (82.6%) patients.

The clinical significance of all obligate anaerobes isolated during this study and the medical management of these patients were also analyzed. These data will be presented elsewhere (4a). However, the results of the anaerobic blood cultures rarely influenced the medical management of these patients, because other cultures (e.g., peritoneal fluid or abscesses) were also positive, the patients were receiving appropriate empiric therapy, or the isolate was judged to be clinically insignificant.

DISCUSSION

In a recent review of bacteremias, Sharp (34) reported that the incidence of anaerobic infections has decreased dramatically. A retrospective review of Sharp's laboratory data collected over a 6-month period established that anaerobes were isolated in only 0.2% of all blood cultures, representing 1.5% of all positive blood cultures. Dorsher and colleagues (4) also examined their experience with anaerobic bacteremia at the Mayo Clinic over a 15-year period and observed a significant decrease in anaerobic infections. Likewise, the data presented in our study demonstrate that the relative incidence of anaerobic bacteremias has steadily decreased during the last 13 years. Whereas the total number of patients with blood cultures which were positive for anaerobes remained relatively constant, the ratio of blood cultures

with anaerobes as a function of the total blood cultures processed dramatically decreased. Dorsher et al. (4) reported that from 1979 to 1988, an average of 4.7 blood cultures per 1,000 total blood cultures were positive for anaerobes and that the average number of anaerobic bacteremias per 100,000 patient-days was 15.1. This is very similar to the experience in our study. Over the 13-year study period, we reported an average of 4.7 positive blood cultures per 1,000 total blood cultures and 12.1 anaerobic bacteremias per 100,000 patient-days.

During the 13-year retrospective review of our blood culture data, we noticed that the incidence of fungemia increased almost 10-fold. This is similar to the experience in other centers in which increased numbers of fungemias have also been observed (6, 12, 17, 20). The increase is in part due to changes in the population of our hospitalized patients (e.g., increased numbers of highly immunocompromised patients, use of broad-spectrum antibacterial agents) as well as to improvements in blood culturing techniques. During the study period we introduced the routine use of biphasic blood culture media, the lysis-centrifugation system, and routine agitation of the biphasic media, all of which have been shown to improve recovery of fungi in blood cultures (3, 5, 10, 11, 15, 22, 28, 33, 38). Although the relative significance of changes in the patient population and in laboratory techniques cannot be delineated, it is clear that documented fungemia is increasing dramatically, particularly in comparison with anaerobic bloodstream infections.

Our analysis of all blood culture isolates for a 12-month period (Table 3) also documents that fungemia (present in 121 patients [268 positive cultures]) is more common than anaerobic bacteremia (present in 82 patients [106 positive cultures]). Furthermore, with the exception of anaerobic bacteria, all major groups of bacteria and fungi were recovered more frequently in aerobic blood culture bottles (Table 3). A total of 21.1% more bacteria and fungi were recovered in biphasic tryptic soy broth bottles than in unvented Columbia bottles. When the data for individual patients were examined, it was found that the organisms were isolated exclusively in anaerobic bottles for only 4.5% of all septic patients, compared with organisms isolated only in aerobic bottles for 12.9% of the patients (Table 4).

With the relative decrease in anaerobic bacteremia and the superior performance of the biphasic bottle compared with the anaerobic bottle, it seems appropriate to question the need for processing blood specimens routinely for anaerobes (34). It has been clearly demonstrated that the success of isolating an organism from blood is directly related to the amount of blood cultured, with between 1.9 and 6.6% more positive blood cultures detected for each additional milliliter of blood processed (8, 14, 18, 31, 35). It has also been shown in this study and others that, with the exception of anaerobic bacteria, many organisms grow preferentially in aerobic blood culture bottles. Blazevic et al. (2) isolated more *Candida*, *Cryptococcus*, *Pseudomonas*, and *Staphylococcus epidermidis* organisms in vented than in unvented Columbia broths; Harkness et al. (9) recovered more *Candida*, *Pseudomonas*, and *Bacillus* species in vented tryptic soy broth bottles; and Tenney et al. (36) recovered more fungi and *Neisseria gonorrhoeae* and *Eubacterium* organisms in vented supplemented peptone broths. In each of these studies more anaerobes were recovered in the unvented blood culture bottles, although the differences between the aerobic and anaerobic bottles were statistically significant in only one study (2). Because the volume of blood cultured directly affects the recovery of bloodstream pathogens, and anaerobic incubation is deleterious to the recovery of fungi and aerobic bacteria, the anaerobic processing of half of the collected blood seems inappropriate. What is unknown, however, is whether the use of two aerobic bottles would increase the overall recovery of organisms or whether the same number of organisms would be recovered, now in two bottles rather than in a single aerobic bottle. Although the studies assessing the effect of culturing a large volume of blood would predict better overall recovery of organisms, resolution of this question will require further comparative testing with one versus two agitated biphasic bottles. It is also important to note that laboratory methodologies, as well as the patient population and antibiotic prescribing practices, can influence overall recoveries of fungi and aerobic and anaerobic bacteria. Other hospitals may not experience the same relative recoveries of these organisms as those reported herein. However, our experience should encourage microbiologists in other hospitals to examine their own experience and weight carefully the advantages and disadvantages of using an anaerobic blood culture bottle.

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