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Evaluation of Routine Subcultures of Macroscopically Negative Blood Cultures for Detection of Anaerobes

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Routine anaerobic subcultures of macroscopically negative blood culture bottles, performed within 1 day of receipt of the culture and after 5 days of incubation, were evaluated. Anaerobes were recovered from 207 (12.3%) of the total 1,688 positive cultures and, of these, 154 were only detected macroscopically, 11 only by subculture, and 42 by both procedures. In no instance was the anaerobe detected earlier with the subculture, and the time required for a definitive identification was reduced for only 10 isolates. Since the subcultures did not significantly improve the detection or early identification of anaerobes, routine anaerobic subcultures are not recommended.

The rapid isolation and accurate identification of microorganisms recovered in blood cultures provide valuable information for the specific therapeutic management of septic patients. Although many organisms can be detected by the production of visible changes in blood culture bottles, such as turbidity, discrete colonies, gas formation, or hemolysis, the aerobic "blind" subculture of macroscopically negative bottles is necessary for the detection of *Haemophilus, Moraxella, Neisseria,* and many isolates of *Pseudomonas* (1-3). In addition, Harkness et al. (4) and Todd and Roe (9) reported that aerobic subcultures performed on the day that the culture was collected resulted in the earlier detection and identification of 48 and 85% of their positive cultures, respectively.

Although anaerobes are frequently recovered in blood cultures (6, 11, 13), the value of blind subcultures for the rapid detection and identification of anaerobes is not known. Bartlett and associates (1) recommended that anaerobic subcultures should be routinely performed after 2 and 5 days of incubation, although we are not aware of any published data which corroborates this recommendation. Hall et al. (3) and Blazevic et al. (2) reported that anaerobic subcultures were not useful, although in the former study anaerobic subcultures were not performed, and in the latter study only 12% of all positive blood cultures were first detected by subculture.

In the study reported herein, we examined the effect of routine anaerobic subcultures on the overall recovery of anaerobes and the time in which the isolates were detected or identified.

MATERIALS AND METHODS

Blood cultures, collected from patients in the Barnes Hospital and the associated Washington University hospitals and clinics, were analyzed during a 19-month period. Blood was inoculated (10%, vol/vol) into one bottle each of tryptic soy broth (Difco Laboratories) and thiol broth (Difco), under vacuum with CO_2 and with 0.025% sodium polyanetholsulfonate. During the first 12 months of the study 50-ml bottles were used, and 100-ml bottles were used during the remainder of the study. The inoculated bottles were immediately transported to the laboratory and, upon receipt, the trypic soy broth bottles were transiently vented with a sterile cotton-plugged needle to release the vacuum. If a fungal infection was suspected, the venting needle was left in place for the duration of the incubation. The bottles were routinely incubated at 35°C for 10 days and were macroscopically examined daily.

During the first 7 months of the study, the blind aerobic and anaerobic subcultures were performed after the cultures had been incubated for at least 24 h and then again after 5 days. During the last 12 months of the study, the first aerobic and anaerobic subcultures were performed on the day the culture was received. A portion of broth was aspirated with a sterile needle and syringe and subcultured onto chocolate blood agar (Difco) and prereduced tryptic soy agar (Difco) supplemented with 5% sheep blood, hemin (5 μg/ml), vitamin K_1 (0.5 μg/ml), cysteine hydrochloride (0.5 mg/ml), and palladium chloride (0.33 mg/ml). The chocolate blood agar and anaerobic sheep blood agar plates were then incubated for 48 h at 35°C in 5% CO_2 in air and an anaerobic atmosphere, respectively. The aerobic and anaerobic subculture plates were examined for bacterial growth after 24 and 48 h of incubation. The day the culture was considered to be positive was when either macroscopic growth

was observed in the culture bottle or growth was observed on the subculture plate, rather than the day when the subculture was performed. If an isolate was detected within 24 h from the time the culture was received in the laboratory, then the time of detection was day 1.

Macroscopically positive cultures were immediately Gram stained, and subcultures were made onto media appropriate for the types of organisms seen in the stained smears. Anaerobes were identified by the antibiotic disk method of Sutter et al. (8), biochemical reactivity was measured with the modified Minitek system described by Stargel et al. (7), and metabolic end product analysis was performed by gas-liquid chromatography (5). Identification of the genera in the family *Bacteroidaceae* was not routinely performed during the first part of this study and, therefore, these isolates will be referred to in the text by the family rather than the specific genus designation.

RESULTS

Between 1 January 1976 and 31 July 1977, 17,314 sets of blood cultures were received. There were 1,688 positive cultures, or 9.7% of the cultures received, and a total of 1,843 organisms were isolated. Anaerobes were recovered in 207 (12.3%) of the 1,688 positive cultures. More than one organism was recovered in 126 cultures, and at least one isolate was an anaerobe in 32 cultures.

The cumulative detection time of the anaerobes is summarized in Table 1. Within the first 2 days of incubation, 43% of the anaerobes were detected, and the mean time of detection was 3.7 days.

The method by which the anaerobes were detected, that is, either by examination of the blood culture bottle, by subculture, or both, is summarized in Table 2. A total of 154 (75%) of the 207 anaerobes were detected by macroscopic examination of the culture bottles alone, 11 (5%) by subculture only, and 42 (20%) by both procedures. Twenty-five percent of the anaerobes were recovered on subculture plates during both the period in which the subcultures were performed after incubation for 1 and 5 days and the period in which they were performed on the day the culture was received and after 5 days.

There were 24 and 29 anaerobes detected with the first and second subcultures, respectively (Table 3). During the period when the first subculture was performed after 24 h of incubation, 10 (12.8%) of the 78 isolates grew on the first subculture plates. When the first subculture was performed on the day the culture was received, 14 (10.9%) of the 129 isolates grew on the subculture plates. Of the 24 anaerobes, 23 detected with the first subculture were also macroscopically detected in the blood culture broths; one isolate, *Propionibacterium*, was detected only on the subculture plates. Thirteen of these 23 anaerobes, including 12 *Bacteroidaceae* and

TABLE 1. *Cumulative detection time of anaerobes in blood cultures*

Organism	No. isolated	Cumulative % positive cultures after days:					Mean (days)
		1	2	3	4	5	
Bacteroidaceae	123	8	40	57	62	72	3.3
Clostridium	28	61	96		100		1.5
Propionibacterium	26		8	12			6.9
Peptostreptococcus	17		62	77	85	92	3.6
Peptococcus	10		10	20	30	50	5.6
Veillonella	3		67		100		2.7
Total	207	13	43	56	61	67[a]	3.7

[a] The remaining 33% of the isolates were detected after 6 to 10 days of incubation.

TABLE 2. *Method of detection of anaerobes in blood cultures*

Organism	No. isolated	No. detected		
		Bottle only	Both bottle and subculture	Subculture only
Bacteroidaceae	123	96	26	1
Clostridium	28	27	1	0
Propionibacterium	26	9	10	7
Peptostreptococcus	17	12	2	3
Peptococcus	10	8	2	0
Veillonella	3	2	1	0

TABLE 3. *Detection of anaerobes with the first and second blind subcultures*

Organism	No. detected with:			
	Subculture 1		Subculture 2	
	Bottle and subculture	Subculture only	Bottle and subculture	Subculture only
Bacteroidaceae	20	0	6	1
Clostridium	1	0	0	0
Propionibacterium	1	1	9	6
Peptostreptococcus	1	0	1	3
Peptococcus	0	0	2	0
Veillonella	0	0	1	0

one *Clostridium*, were macroscopically detected 1 day before growth on the subculture plate was observed. Growth on the subculture plate and in the culture broths was detected simultaneously for the other 10 anaerobes.

DISCUSSION

In the study reported herein, anaerobes were recovered in 12.3% of the 1,688 positive cultures received during a 19-month period, which is an incidence similar to that reported by others (2, 10). Although it is desirable to rapidly detect and identify anaerobes from blood cultures, blind anaerobic subcultures that were performed as described herein did not significantly improve the overall recovery of anaerobes or the rate in which the isolates were detected.

Of the total 207 anaerobes isolated in this study, 11 (5.3%) would not have been detected without the blind subcultures. One (*Propionibacterium*) of the 11 anaerobes was only detected with the first subculture, and 10 anaerobes (6 *Propionibacterium*, three *Peptostreptococcus*, and one *Bacteroidaceae*) were only detected with the second subculture. Each of the anaerobes was the only organism recovered from separate patients. After a review of the medical record of each patient, either the 11 isolates were considered to represent skin contaminants, or their significance could not be clearly determined because of the complex clinical course of the patient and the empirical use of broad-spectrum antimicrobial therapy.

Anaerobic subcultures, performed either on the day when the culture was received or after the culture was incubated for 24 h, did not improve the rate in which anaerobes were detected. None of the isolates was detected earlier with the first subculture when compared with examination of the culture bottles.

During the 19-month period reported herein, 10 anaerobes were detected on the same day with the first subculture and by macroscopic examination of the culture bottles. The lack of growth on the aerobic subculture plates provided preliminary information that these 10 isolates were anaerobes and, because the isolates had grown on the anaerobic subculture plates, the time required for the definitive identification was reduced. It is possible, however, to presumptively identify a blood culture isolate as an anaerobe without performing the blind anaerobic subcultures. *Bacteroidaceae* and *Clostridium* were the most frequently isolated anaerobes in this study. The detection of pleomorphic or fusiform gram-negative bacilli in the blood culture broth is suggestive of *Bacteroidaceae*, and the detection of gram-positive bacilli in a culture with hemolysis and abundant gas production is suggestive of *Clostridium*. In addition, the direct analysis of the blood culture broths for volatile fatty acids by gas-liquid chromatography can provide an accurate preliminary identification of the genus of the anaerobic isolate (12; J. E. Sondag, P. R. Murray, and M. L. Heath, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C182, p. 307).

The timing of the aerobic subcultures performed in this study was selected to maximize the early detection and identification of aerobic blood culture isolates (4, 9). Anaerobic subcultures were performed at the same time to reduce both the time necessary to process the cultures and the possibility of contaminating the cultures during multiple entries into the bottles. Before August 1976, the first aerobic and anaerobic subcultures were performed after incubation for 24 h. However, this delayed the detection and identification of aerobic isolates, particularly gram-negative bacilli, as was reported by others (4, 9). The percentage of anaerobes recovered with the subculture made after 24 h was similar to that with the same-day subcultures, namely, 12.8 and 10.9%, respectively. If anaerobic subcultures were delayed for 24 or 48 h (1), the identification of those anaerobes which had grown on the early subculture plates would be delayed.

Finally, the expense involved in performing

anaerobic subcultures is not trivial. In this laboratory, approximately 11,000 cultures are processed in a year, and each culture includes two bottles of broth which are subcultured twice. A total of approximately 44,000 anaerobic subcultures are, therefore, performed in a year. In this laboratory, two subcultures are inoculated onto a single anaerobic plate, each of which costs $0.35. The cost for media alone is, therefore, $7,700 per year. Other less tangible factors such as technician time significantly increase this expense.

In summary, although anaerobes were recovered from 12.3% of the positive cultures, routine anaerobic subcultures of macroscopically negative blood culture bottles cannot be recommended when performed at the time periods described above. Blind anaerobic subcultures did not significantly improve the overall recovery or early detection and identification of anaerobic blood culture isolates.

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