JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1978, p. 764-766
0095-1137/78/0008-0764$02.00/0

Vol. 8, No. 6
Printed in U.S.A.

Evaluation of a Routine Anaerobic Subculture of Blood Cultures for Detection of Anaerobic Bacteremia

JOHN W. PAISLEY,† JON E. ROSENBLATT, MARSHA HALL, AND JOHN A. WASHINGTON II*
Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received for publication 18 September 1978

The value of a routine 48-h anaerobic subculture of blood cultures was assessed in our laboratory over a 4-month period. Excluding presumed contaminants, anaerobes represented 51 (6.9%) of the total number of 734 positive cultures. Sixteen isolates (all *Bacteroides*) from six patients were detected by the anaerobic subculture. All but one of these were also detected macroscopically. Excluding the one isolate, the routine anaerobic subculture hastened the identification of anaerobic organisms by only 1 day in two patients. We conclude that a routine anaerobic subculture is not indicated for the detection of anaerobic bacteremia.

The value, timing, and number of aerobic blood subcultures have been well established; however, similar data regarding anaerobic subcultures is scarce (7, 11). In a survey of blood culture practices in 21 laboratories, 12 regularly performed anaerobic subcultures of all macroscopically negative blood culture bottles without macroscopic evidence of growth (1). Although many recent studies of blood culture procedures have included routine anaerobic subcultures, the yield from this procedure has not been evaluated (4, 6, 8, 11). The lack of information on this subject may account for the conflicting recommendations regarding the need for this procedure in detecting anaerobic bacteremia (2, 3, 12). This report presents the results of a 4-month evaluation of routine anaerobic subcultures.

Blood collected aseptically by a venipuncture team from patients with suspected bacteremia was inoculated (10%, vol/vol) into three bottles. Two bottles contained 100 ml of tryptic soy broth with 0.025% sodium polyanetholsulfonate under vacuum with CO_2 (Difco Laboratories, Detroit, Mich.). During the early part of the study period, the third bottle contained 100 ml of Trypticase soy broth with 0.025% sodium polyanetholsulfonate under vacuum with CO_2 (Bioquest, Cockeysville, Md.); during the latter part, it contained 100 ml of tryptic soy broth with 0.025% sodium polyanetholsulfonate and 15% sorbitol under vacuum with CO_2 (Difco Laboratories). Upon receipt in the laboratory, one of the two bottles with tryptic soy broth and the third bottle were transiently vented. All bottles were then incubated at 35°C and examined macroscopically later on the day of receipt, once daily for 7 days, and finally on day 14 of incubation.

Routine subcultures to chocolate blood agar plates of macroscopically negative bottles were performed on the day of receipt and again after 48 h of incubation (7, 11). The plates were incubated at 35°C in an atmosphere containing 10% CO_2 and were examined after 24 and 48 h. Subcultures of suspected (macroscopically) positive blood culture bottles were made to media selected on the basis of the organism's Gram-stained morphology; this always included an anaerobically incubated blood agar plate.

From 18 October 1977 to 18 February 1978, a routine anaerobic subculture of all macroscopically negative bottles was performed in addition to the aerobic subculture at 48 h. Samples of each bottle were withdrawn with a sterile needle and syringe and inoculated onto sheep blood agar plates. After each plate was inoculated, it was immediately placed in an anaerobic jar (GasPak; Bioquest) which had been initially flushed and then maintained under a continuous flow of CO_2. When each jar was filled with plates, strict anaerobic conditions were achieved by using the GasPak system. The plates were incubated at 35°C and then transferred to an anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, Mich.), where they were examined for evidence of growth at 16 to 20 h after inoculation and again at 40 to 48 h.

All isolates were identified in the Anaerobe Laboratory of the Section of Clinical Microbiology using previously described methods (9). To compare the times required for organism detection, bottles with an anaerobe isolated on subcultures were reincubated until macroscopic evidence of growth was present, up to a total of 14 days.

† Present address: Department of Pediatrics, Denver General Hospital, Denver, CO 80204.

During the study period, 983 blood cultures were positive (11.7% cultures representing 707 patients) out of 8,384 obtained. A total of 125 cultures yielded anaerobes, representing 1.5% of the total number of cultures taken and 12.7% of the positives. When single cultures of *Staphylococcus epidermidis, Corynebacterium* spp., *Bacillus* spp., and *Propionibacterium acnes* were excluded as probable contaminants, there was a total of 734 (8.7%) positive cultures; anaerobes represented 0.61% of the total number of cultures drawn and 6.9% of the total number of positives.

The time to detection of the isolates, macroscopically and/or by the 48-h subculture, is presented in Table 1. Five or more days were required for detection of 72 of 74 (97%) *P. acnes* isolates. Three days or less were required for the detection of 45 of 51 (88%) of the remaining and more probably clinically significant isolates.

The method of initial detection is presented in Table 2. If growth was observed simultaneously on the early subculture plate and macroscopically in the bottle, the subculture was considered the method of initial detection. Excluding *P. acnes,* 16 of 51 (31%) anaerobes were first detected by the anaerobic subculture, including 16 of 35 (46%) *Bacteroides.* Thirteen of these 16 isolates were detected after 16 to 20 h of incubation of the anaerobic subculture plate. A single isolate of *Bacteroides corrodens* was detected only by the anaerobic subculture. All other isolates were detected macroscopically only or by both methods.

Although the data suggest that the anerobic subculture is useful, its utility is more apparent than real if the data are analyzed by patients rather than by cultures. There were 28 patients with isolates other than *P. acnes.* The 16 isolates of *Bacteroides* detected by the anaerobic subculture were obtained from only six patients. Of these six patients, three had the same organism detected macroscopically in another blood culture 1 day before detection by the anaerobic subculture. Two other patients' cultures were macroscopically positive on the same day the isolate was detected on the anaerobic subculture. The final patient's isolate (*B. corrodens*) was detected only on the anaerobic subculture. Excluding the latter patient, therefore, identification of an anaerobic blood isolate was hastened by only 1 day in two patients by use of the anaerobic subculture.

The results of this study suggest that a routine 48-h anaerobic blood culture is of little value in the detection of anaerobic isolates in our laboratory. Although the small number of certain species isolated precludes generalizations, the anaerobic subculture specifically did very little to hasten the detection of *Bacteroides fragilis* and *Clostridium* sp., the most frequent clinically

TABLE 2. *Method of initial detection of 125 anaerobic blood isolates*

Strain	Macroscopic	48-h anaerobic subculture
Anaerobic gram-positive cocci	3	—
Clostridium spp.	6	—
P. acnes	74	—
Non-sporeforming gram-positive bacilli[a]	4	—
Bacteroides fragilis group	19	15
B. corrodens	—	1
Fusobacterium spp.	3	—

[a] Excluding *P. acnes.*

TABLE 1. *Incubation time required for detection, macroscopically and/or by 48-h subculture, of 125 anaerobic blood isolates*

Strain	No. of isolates recovered after incubation (days):							
	1	2	3	4	5	6	7	>7
Anaerobic gram-positive cocci		1	1				1	
Clostridium spp.	2	3		1				
P. acnes		1		1	3	12	12	45
Non-sporeforming gram-positive bacilli[a]		4						
Bacteroides fragilis	2	12	17	2	1			
B. corrodens				1				
Fusobacterium spp.		2	1					
Cumulative number of isolates positive (%[b])	4 (3/8)	27 (22/51)	46 (37/88)	51 (41/96)	55 (44/98)	67 (54/98)	80 (64/100)	125 (100/—)

[a] Excluding *P. acnes.*

[b] Cumulative percent positive/cumulative percent positive excluding *P. acnes.*

significant anaerobes isolated from blood in this and other studies (3, 4, 5, 8, 10, 13).

In a strict sense, these results apply only to the blood culture protocol used during this study. Our conclusion, however, is similar to that of Blazevic et al., who performed routine anaerobic subcultures after 1 and 4 days of incubation (3). Routine anaerobic subcultures require additional time and materials, both of which are expensive and neither of which is particularly productive in terms of increasing the yield or reducing the time interval required for detecting the presence of anaerobes. Recommendations regarding the necessity of routine anaerobic subcultures should be revised.

LITERATURE CITED

1. **Bartlett, R. C.** 1973. Contemporary blood culture practices, p. 15-35. *In* A. C. Sonnenwirth (ed.), Bacteremia: laboratory and clinical aspects. Charles C. Thomas Publisher, Springfield, Ill.
2. **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.
3. **Blazevic, D. J., J. E. Stemper, and J. M. Matsen.** 1974. Comparison of macroscopic examination, routine Gram stains, and routine subcultures in the initial detection of positive blood cultures. Appl. Microbiol. **27**:537-539.
4. **Ellner, P. D., T. E. Kiehn, J. L. Beebe, and L. R. McCarthy.** 1976. Critical analysis of hypertonic medium and agitation in detection of bacteremia. J. Clin. Microbiol. **4**:216-224.
5. **Hall, M., E. Warren, and J. A. Washington, II.** 1974. Comparison of two liquid blood culture media containing sodium polyanetholsulfonate: tryptic soy and Columbia. Appl. Microbiol. **27**:699-702.
6. **Hall, M., E. Warren, and J. A. Washington, II.** 1974. Detection of bacteremia with liquid media containing sodium polyanetholsulfonate. Appl. Microbiol. **27**:187-191.
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. **Mangels, J. I., L. H. Lindberg, and K. L. Vosti.** 1977. Comparative evaluation of three different commercial blood culture media for recovery of anaerobic organisms. J. Clin. Microbiol. **5**:505-509.
9. **Rosenblatt, J. E.** 1976. Isolation and identification of anaerobic bacteria. Human Pathol. **7**:177-186.
10. **Rosner, R.** 1976. Growth patterns of a wide spectrum of organisms encountered in clinical blood cultures using both hypertonic and isotonic media. Am. J. Clin. Pathol. **65**:706-710.
11. **Todd, J. K., and M. H. Roe.** 1975. Rapid detection of bacteremia by an early subculture technique. Am. J. Clin. Pathol. **64**:694-699.
12. **Washington, J. A., II.** 1978. Conventional approaches to blood culture, p. 41-87. *In* J. A. Washington II (ed.), The detection of septicemia. CRC Press, Inc., West Palm Beach, Fla.
13. **Wilson, W. R., W. J. Martin, C. J. Wilkowske, and J. A. Washington, II.** 1972. Anaerobic bacteremia. Mayo Clin. Proc. **47**:639-646.