JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1979, p. 88-92
0095-1137/79/01-0088/05$02.00/0

Vol. 9, No. 1

Evaluation of Blood Culture Procedures in a Pediatric Hospital

ELIZABETH G. SZYMCZAK,[2] JUDITH T. BARR,[3] WILLIAM A. DURBIN,[1] AND DONALD A. GOLDMANN[1]*

Division of Infectious Diseases[1] and Bacteriology Laboratory,[2] Children's Hospital Medical Center, Boston, Massachusetts 02115, and Medical Laboratory Science Program, Northeastern University, Boston, Massachusetts 02115[3]

Received for publication 8 November 1978

To determine optimal clinical laboratory techniques for detecting pediatric bacteremia, we studied 7,768 consecutive blood cultures in a 1-year period. Blood was inoculated into one vented 50-ml bottle of brucella broth with 0.05% sodium polyanetholsulfonate and one unvented 50-ml bottle of Columbia broth with 0.05% sodium polyanetholsulfonate and 0.05% cysteine. Bottles were visually examined for growth on days 1 through 7 and blindly subcultured aerobically and anaerobically on days 1, 2, and 7. There were 724 (9.3%) positive cultures, and 484 (6.2%) were clinically significant. The most frequent isolates from bacteremic patients were *Haemophilus influenzae* (24%) and *Streptococcus pneumoniae* (17%). Growth was noted in only one bottle in 25% of clinically significant isolates. Bottles inoculated with ≥1 ml of blood became positive earlier than bottles inoculated with <1 ml. After 1 day of incubation, 48% of the clinically significant cultures showed growth on visual examination, whereas 85% showed growth on subculture. Only 19% of *Haemophilus* isolates were detected visually on day 1, whereas 88% were recovered on subculture. By day 7, 3.5% of all isolates (including 18% of pneumococcal isolates and 1% of *Haemophilus* isolates) could no longer be recovered on subculture. We conclude that a two-bottle blood culture system and blind subculture within 24 h will optimize detection of pediatric bacteremia.

Prompt detection of microorganisms in blood cultures is one of the most important responsibilities of clinical microbiology laboratories. Although blood culture methodology tailored to adult populations has been reviewed extensively (1, 7), children pose unique problems. The spectrum of organisms causing childhood bacteremia is different than that causing the disease in adults. For example, *Haemophilus influenzae*, a rather fastidious pathogen, is a frequent isolate from bacteremic children but rarely produces adult disease (4). The recommended 5 to 10 ml of blood per culture often cannot be obtained from infants and young children, and it has been suggested that this may delay recognition of positive blood cultures. Moreover, cultures inoculated with small volumes of blood might fail to detect bacteremia altogether since the concentration of organisms in the blood of bacteremic children is occasionally very low (3).

We have evaluated the ability of recommended blood culture procedures to detect pediatric bacteremia and have determined an appropriate protocol for visual examination and subculture.

MATERIALS AND METHODS

Collection techniques. All blood cultures submitted to the Clinical Microbiology Laboratory, Children's Hospital Medical Center, Boston, Mass. from July 1976 to June 1977 were studied. Cultures were collected by pediatric house officers and the phlebotomy team following hospital protocol. The skin was decontaminated with povidone-iodine followed by 70% isopropyl alcohol. Tops of culture bottles were also decontaminated with povidone-iodine and alcohol and were allowed to dry before being inoculated. Bottles were inoculated at bedside.

A minimum inoculum of 1 ml for each of the two blood culture bottles was recommended, and 5 ml was requested. Inoculum volume was recorded at bedside and verified when cultures were received in the laboratory.

Routine blood culture procedures. Routine blood culture media consisted of one 50-ml bottle of brucella broth with 0.05% sodium polyanetholsulfonate (Pfizer Inc.) and one 50-ml bottle of Columbia broth with 0.05% sodium polyanetholsulfonate and 0.05% cysteine (Pfizer). Both bottles were partially evacuated and had an atmosphere of 5% CO_2. The inoculated brucella broth bottle was vented upon receipt in the laboratory with a sterile cotton-plugged needle (Sherwood). Both bottles were incubated at

35°C and inspected daily for 7 days for evidence of macroscopic growth. In addition, blind aerobic and anaerobic subcultures of macroscopically negative cultures were performed on days 1, 2, and 7 of incubation. A disposable 3-ml syringe (Becton, Dickinson & Co.) was used to remove 0.5 ml from the Columbia broth, and a sterile double-ended needle (Sherwood) was used to remove approximately 0.1 ml from the brucella broth. Approximately 0.1 ml of broth from each bottle was inoculated on a chocolate agar plate (Grand Island Biological Co.). Blind brucella broth subcultures were incubated at 35°C in 6% CO_2 and examined at 24 and 48 h for growth. Blind Columbia broth subcultures were incubated in GasPak jars (Baltimore Biological Laboratory) at 35°C and examined at 48 h.

Quantitative blood cultures. In addition to collecting blood for routine culture bottles, physicians obtained another 1 ml, when readily available, for quantitative culture. Quantitative cultures were performed as described previously (3). Briefly, blood was inoculated into a sterile tube containing 1 ml of brucella broth with 0.05% sodium polyanetholsulfonate. Inoculated tubes were transported at room temperature and processed within 30 min. Of the 2 ml in the tube, 1 ml was added to 20 ml of melted brain heart infusion agar (Scott Laboratories, Inc.) containing 0.5 ml of IsoVitaleX (Baltimore Biological Laboratory), 0.001 ml from the quantitative tube was added to 20 ml of melted brain heart infusion agar containing 0.5 ml of IsoVitaleX and 0.2 ml of defibrinated horse blood (Grand Island Biological Co.), and the remainder was plated directly on a chocolate agar plate. Plates were examined daily for four days, and mean colony counts were determined on day 4.

Positive blood cultures. Culture bottles with visual evidence of growth (turbidity, gas production, hemolysis) were Gram stained and subcultured aerobically and anaerobically to chocolate agar and various selective and differential media according to Gram stain. Preliminary antibiotic susceptibility testing was performed by using standard methods (2), which were modified by using approximately 0.2 ml of broth from the blood culture bottle as the initial inoculum. Identification and susceptibility testing were repeated by using standard methods when the organism was isolated on solid media. Microorganisms isolated from routine blind subcultures were examined in the same manner. Positive culture bottles were reincubated and subcultured to chocolate agar on day 7. When endocarditis, brucellosis, or fungal infection were suspected, bottles were subcultured as above and after 14 days of incubation.

Data collection. The following information was recorded for all cultures: age and hospital location of patient, antibiotic therapy in the previous 24 h, inoculum volume, number of positive cultures per bacteremic episode, organisms isolated, time and method of detection, aerobic and/or anaerobic growth, and the 1-, 2-, and 7-day subculture results. All positive cultures were evaluated for clinical significance by the Infectious Disease Service. Isolates were considered clinically significant if the patient's course was compatible with blood stream infection or if the same organism was isolated from two or more blood cultures within 24 h.

RESULTS

Quantitative culture results confirmed the sensitivity of the routine blood culture procedure in detecting bacteremia. Only 1 of 24 positive quantitative cultures was not positive by the routine method, and this patient had already been started on antibiotic therapy. The two-bottle method detected very low numbers of organisms; for example, the density of organisms in the nine cases of pneumococcemia detected by both routine and quantitative cultures ranged from 0.5 to 200 colony-forming units per ml (mean 51 colony-forming units per ml).

Of the 7,768 blood cultures examined, 724 (9.3%) were positive, and 484 (6.2%) grew clinically significant organisms. The clinically significant isolates were obtained from 295 patients, giving a mean of 1.6 positive cultures per episode of bacteremia. Cultures taken on the day of admission were positive in 55% of bacteremic patients, and cultures obtained within 48 h of admission were positive in an additional 9%. In 30% of patients, the first positive culture was obtained more than 2 days after admission. One percent of bacteremic patients were treated as outpatients, and 4% were recalled for admission because of positive cultures in the emergency room.

Microorganisms isolated. *H. influenzae* (24.1% of patients) and *Streptococcus pneumoniae* (17.3% of patients) were the most frequent clinically significant isolates, followed by *Staphylococcus aureus* (13.6%) and *Escherichia coli* (9.8%) (Table 1). *Haemophilus* and pneumococcal bacteremia tended to occur in patients less than 5 years old, whereas staphylococcal and *E. coli* bacteremia were relatively more frequent in children more than 5 years old and in neonates (Table 2). Almost all *H. influenzae* and *S. pneumoniae* bacteremias (100% and 98%, respectively) were detected by cultures obtained within 48 h of admission, whereas the majority of *S. aureus* and *E. coli* bacteremias (53% and 66%, respectively) were detected after 2 days in the hospital. *Staphylococcus epidermidis* (53.2% of patients), viridans streptococci (10.4%), *Corynebacterium* sp. (8.1%), and *Proprionibacterium acnes* (7.6%) were the most frequent nonclinically significant isolates (Table 3).

Visual examination versus blind subculture. Visual examination of cultures within 24 h of receipt in the laboratory detected only 48% of clinically significant isolates, whereas blind subculture within 24 h detected 85% of clinically significant isolates (Fig. 1). By day 2, 96% of clinically significant isolates had been detected by subculture. By day 3, all but 1% had been detected by either visual examination or subcul-

TABLE 1. *Spectrum of clinically significant organisms*

Organism	No. of patients
H. influenzae	71 (24.1)[a]
S. pneumoniae	51 (17.3)
S. aureus	40 (13.6)
E. coli	29 (9.8)
S. epidermidis	28 (9.5)
Klebsiella pneumoniae	12 (4.0)
Viridans streptococci	9 (3.1)
Candida sp.	7 (2.4)
Salmonella sp.	7 (2.4)
Streptococcus group A	6 (2.0)
Enterobacter cloacae	5 (1.7)
Streptococcus group B	4 (1.3)
Enterococcus	4 (1.3)
Pseudomonas aeruginosa	4 (1.3)
Aeromonas hydrophila	2 (0.7)
Bacteroides sp.	2 (0.7)
Neisseria meningitidis	2 (0.7)
Proteus morganii	2 (0.7)
Miscellaneous[b]	10 (3.4)

[a] Numbers in parentheses are percentages of total.

[b] Includes one isolate each of *Acinetobacter anitratus, Bacillus* sp., *Citrobacter freundii, Corynebacterium* sp., *Enterobacter agglomerans, Listeria monocytogenes, Neisseria gonorrhoeae, Serratia marcescens, Streptococcus* group C, and *Streptococcus* group G.

TABLE 2. *Age at onset of bacteremia*

Organism	No. of patients in the following age groups:			
	<1 month	1-5 months	6 months-5 years	>5 years
H. influenzae	2	13	53	3
S. pneumoniae	1	9	32	9
S. aureus	6	4	6	24
E. coli	10	7	6	6

TABLE 3. *Spectrum of contaminants*

Organism	No. of patients
S. epidermidis	118 (53.2)[a]
Viridans streptococci	23 (10.4)
Corynebacterium sp.	18 (8.1)
P. acnes	17 (7.6)
Aspergillus sp.	15 (6.7)
Bacillus sp.	10 (4.5)
Micrococcus sp.	7 (3.2)
Enterococcus	5 (2.3)
S. aureus	4 (1.8)
Enterobacter cloacae	2 (1.0)
Klebsiella pneumoniae	1 (0.4)
E. coli	1 (0.4)
Clostridium sp.	1 (0.4)

[a] Numbers in parentheses are percentages of total.

ture. The 7-day blind subculture first detected only two isolates of *H. influenzae* and one isolate of *Neisseria meningitidis.*

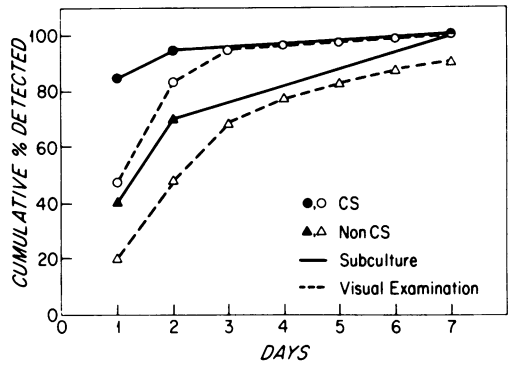

FIG. 1. *Visual versus subculture detection of growth. CS, Clinically significant.*

Only 19% of *H. influenzae* isolates were visually detectable at 24 h, whereas 88% were positive by day 1 subculture. Routine subculture detected growth that was often not visible even after the blind subculture had become positive. Of 245 cultures that were visually negative after 24 h but were positive by day 1 subculture, 11% were still visually negative at 48 h; 39% of these isolates were *H. influenzae*, and 21% were *S. pneumoniae.*

Visual examination at 24 h detected 13% of contaminants, whereas day 1 subculture detected 46%. A total of 56% of *S. epidermidis* isolates were recovered from the day 1 subculture, and 49% of *Corynebacterium* sp. and *Proprionibacterium* sp. isolates were recovered from the day 7 subculture. One-third of *Corynebacterium* sp. and *Proprionibacterium* sp. isolates were never detected by visual examination.

Recovery of microorganisms from vented and unvented bottles. Only 75% of clinically significant strains were isolated from both bottles of the two-bottle blood culture set (Table 4). Growth was noted only in the vented bottle in 14% and only in the unvented bottle in 11% of clinically significant isolates. A total of 46% of pneumococcal isolates grew in only one bottle. *Neisseria* sp. and *Candida* sp. were recovered most frequently from the vented bottle. A total of 70% of contaminants were recovered from only one bottle (40% from the vented bottle and 30% from the unvented bottle).

By day 7, 3.5% of isolates, including 18% of pneumococcal isolates and 1% of *Haemophilus* isolates, could no longer be recovered on subculture. In the majority of cases (13/17), organisms were no longer viable in the unvented bottle.

Effect of inoculum volume on detection time. Both vented and unvented bottles were inoculated with the recommended ≥1 ml of blood in 89% of cultures, but only 8% were inoculated with ≥5 ml. Bottles inoculated with

TABLE 4. *Recovery of clinically significant organisms from vented and unvented bottles*

Organism	% Of organisms recovered from:		
	Both bottles	Vented bottles only	Unvented bottles only
S. pneumoniae	54	26	20
H. influenzae	86	8	6
Staphylococcus sp.	81	11	8
Streptococcus sp.	84	6	10
Candida sp.	32	58	10
Neisseria sp.	25	75	0
Enteric gram-negative bacilli	80	10	10
Nonenteric gram-negative bacilli	80	20	0
Bacteroides sp.	0	0	100
Total of all organisms	75%	14%	11%

≥1 ml of blood showed growth earlier than did bottles inoculated with <1 ml (Table 5). Inoculation with ≥5 ml of blood increased recovery slightly in the unvented bottle but not in the vented bottle. Small inocula were not associated with growth in only one bottle: 11% of cultures in which only one bottle showed growth were inoculated with <1 ml, as were 11% of cultures in which both bottles showed growth.

TABLE 5. *Effect of inoculum volume on detection time*

Vol (ml)	% Detected on day 1
Vented bottle	
<1	55.1 } [a]
≥1	67.1 } [a]
1–4.9	67.3
≥5	65.8
Unvented bottle	
<1	51.4
≥1	59.7
1–4.9	59.2
≥5	64.2

[a] $P < 0.05$ by the chi-square test.

DISCUSSION

We have found that the spectrum of organisms causing bacteremia in children is very different than that causing the disease in adults (4). *H. influenzae* and *S. pneumoniae* are the pathogens most frequently isolated from the blood of bacteremic patients at our institution. Bacteremia in young children is particularly likely to be due to these organisms. Similar findings were reported in a recent study of hospitalized children (8). Since our study included patient samples collected in clinics and the emergency room, we were able to confirm that *Haemophilus* and pneumococcal bacteremia occur primarily in outpatients. In contrast, bacteremia caused by *S. aureus* or *E. coli* more frequently affects hospitalized patients, primarily neonates and older children.

The frequency with which *Haemophilus* is isolated from the blood of bacteremic children has important implications for clinical laboratory practice. We have found that reliance on visual inspection of blood culture bottles can significantly delay the detection of bacteremia, particularly when the pathogen is *H. influenzae*. Visual examination within the first 24 h of incubation detected bacterial growth in only 19% of cultures positive for *H. influenzae*. Even after 48 h, culture bottles often showed no visual evidence of growth. In contrast, 88% of *Haemophilus* isolates were detected by day 1 subculture. Thus, reliance on visual detection of growth is likely to delay the diagnosis of bacteremia in young children in whom *Haemophilus* is such a frequent pathogen; first day subculture is critical.

In addition to permitting early detection of positive cultures, first day subculture provides discrete colonies for identification and antibiotic susceptibility testing procedures. When laboratory staffing permits, other investigators have suggested that subculturing can be performed profitably as early as 4 h after culture bottles are inoculated (6). Isolation, identification, and susceptibility testing can be further accelerated by setting up quantitative cultures at the same time that routine blood cultures are obtained (3).

There is another compelling reason to subculture bottles before more than 1 or 2 days have elapsed: it may be impossible to recover pathogenic bacteria if incubation is prolonged. We found that 18% of pneumococcal isolates and 1% of *Haemophilus* isolates could no longer be subcultured after 7 days of incubation; this was particularly likely to be a problem with the unvented bottle. On the other hand, contaminants such as *Corynebacterium* sp. and *P. acnes* were more likely to be recovered from day 7 subcultures than from day 1 or day 2 subcultures.

A total of 25% of our clinically significant isolates grew in only one bottle. This was not primarily due to the known preference of *Candida* and *Pseudomonas* for vented bottles and of anaerobic bacteria for unvented bottles. Rather, facultative organisms, particularly *S. pneumoniae*, frequently grew in only one bottle. It is, therefore, important to perform routine aerobic subcultures of both the unvented and vented bottles. Routine anaerobic subcultures appear to be less rewarding; we noted only one case of anaerobic bacteremia during our study, and growth was detected by visual examination.

Pediatric patients pose a unique problem since it is often impossible to obtain a large volume of

blood for culture. Our studies augment previous reports suggesting that recovery of organisms may be impaired if small volumes of blood are inoculated into culture bottles (5). It is particularly likely that pediatric bacteremia will be missed if less than 1 ml of blood is inoculated. This is not surprising since we have found that the density of bacteria in the blood of pediatric patients may be very low. In our previous investigation of quantitative blood cultures, we reported a case of pneumococcemia with 0.5 colony-forming units per ml of blood (3). Since publication of that report, we have noted a case of *Haemophilus* bacteremia secondary to epiglottitis in which the density of bacteria in the blood was also 0.5 colony-forming units per ml.

In conclusion, we have found that use of a two-bottle blood culture system is a sensitive method for the detection of pediatric bacteremia, provided that a sufficient volume of blood (at least 1 ml) is inoculated. Visual examination with subculture only on day 7 or on days 2 and 7 is not a satisfactory protocol for the detection of bacteremia in children. Reliance on visual examination is particularly inappropriate for the detection of *H. influenzae*. Rather, blind subculture should be performed during the first 24 h of incubation.

ACKNOWLEDGMENTS

We thank the technologists of the Children's Hospital Bacteriology Laboratory for their help in performing this study. We also thank Ann Macone for her suggestions and assistance and Jennifer Clarke for computerizing our data.

LITERATURE CITED

1. **Bartlett, R. C., P. D. Ellner, and J. A. Washington II.** 1974. Cumitech 1, Blood cultures. J. C. Sherris (coordinating ed.). American Society for Microbiology Washington, D.C.
2. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. **45**:493–496.
3. **Durbin, W. A., E. G. Szymczak, and D. A. Goldmann.** 1978. Quantitative blood cultures in childhood bacteremia. J. Pediatr. **92**:778–780.
4. **Finegold, S. M.** 1973. Early detection of bacteremia, p. 36–46. *In* A. C. Sonnenwirth (ed.), Bacteremia: laboratory and clinical aspects. Charles C Thomas, Publisher, Springfield, Ill.
5. **Hall, M., E. Warren, and J. A. Washington II.** 1974. Detection of bacteremia with liquid media containing sodium polyanetholsulfonate. Appl. Microbiol. **27**: 187–191.
6. **Todd, J. K., and M. H. Roe.** 1975. Rapid detection of bacteremia by an early subculture technique. Am. J. Clin. Pathol. **64**:694–699
7. **Washington, J. A., II.** 1975. Blood cultures: principles and techniques. Mayo Clin. Proc. **50**:91–98.
8. **Winchester, P. D., J. K. Todd, and M. H. Roe.** 1977. Bacteremia in hospitalized children. Am. J. Dis. Child. **131**:753–758.