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Recovery of *Haemophilus influenzae* from Twenty-Three Blood Culture Media

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Haemophilus influenzae is an important agent of bacteremia and has fastidious growth requirements. The purpose of this investigation was to determine the ability of commercial blood culture media to support the growth of this fastidious microorganism. Twenty-three types of blood culture media were inoculated with individual suspensions of eight strains of *H. influenzae* in the presence or absence of an erythrocyte-serum mixture. The rates of recovery of the *H. influenzae* strains from the various types of blood culture media were compared. The results demonstrated that the type of medium, the manufacturer, the erythrocyte-serum mixture, and the strain of *H. influenzae* influenced the recovery rates of *H. influenzae*. Optimal recovery of the strains of *H. influenzae* was obtained from brain heart infusion blood culture medium (GIBCO). Tryptic soy broth (GIBCO) and supplemental peptone of Becton, Dickinson and Co. also were found to be superior to the remaining types of media tested for the recovery of *H. influenzae*.

Culture of blood provides valuable help in establishing the etiological agent of bacteremia (8, 17, 20, 23). In a previous investigation (1) we have shown that the recovery of microorganisms from commercial blood culture media was dependent upon the type and source of medium and the genus of the isolate. The microorganism that the blood culture media most frequently failed to recover was *Haemophilus influenzae;* only one strain (type B) was examined, however (1). Although similar difficulties in isolating *H. influenzae* from blood culture media have been reported (5, 8, 15, 18, 21, 22), only a few studies have shown that the type of medium influenced the recovery rate of *H. influenzae* (5, 18, 23). Since *H. influenzae* has fastidious growth requirements (8) and is an important etiological agent of bacteremia (4, 10-12, 18), the purpose of this investigation was to determine the ability of different types of blood culture media to support the growth of a number of strains of *H. influenzae*. Twenty-three blood culture media were inoculated with individual strains of *H. influenzae* in the presence or absence of an erythrocyte-serum mixture (RBC-SM). The recovery rates of *H. influenzae* from the types of blood culture media were compared.

MATERIALS AND METHODS

Blood culture media. Twenty-three blood culture media were purchased from seven commercial manufacturers. Blood culture bottles contained approximately 50 ml of medium supplemented with either sodium polyanethol sulfonate or sodium amylosulfate. The concentrations of these supplements ranged from 0.025 to 0.05%.

Microorganisms. The strains of *H. influenzae* prepared for use as stock cultures in this investigation are listed in Table 1. Seed cultures were prepared by inoculating brain heart infusion (BHI) broth with a single colony obtained from a recent clinical isolate (blood, spinal fluid, etc.), and used for inoculating separate 100-ml portions of BHI broth. The BHI broth was supplemented with coenzyme, vitamins, and amino acids enrichment (CVA; GIBCO). The cultures were incubated at 35°C for 18 h and centrifuged at 10,000 rpm for 20 min. The pellets were suspended and washed three times with fresh BHI broth. After the final centrifugation, the pellets were suspended in 20 ml of BHI broth, and 2-ml samples of each strain of *H. influenzae* were placed in vials, sealed, and stored in liquid nitrogen.

Determination of the number of strains of *H. influenzae* used for inoculation of blood culture media. Frozen vials containing the strains of *H. influenzae* were thawed, and the bacterial suspensions were serially diluted to yield a final concentration of 10 to 17 microorganisms per ml (Table 1). An aliquot of the inoculum (0.5 ml) was plated on chocolate agar plates to determine the actual number of microorganisms introduced into each blood culture medium. Colonies were counted 24 h after incubation at 35°C, and the number of microorganisms per milliliter of inoculum was determined. Preliminary experiments showed no difference in the growth of *H. influenzae* in the blood culture media inoculated with cultures obtained from the frozen vials or grown in broth.

TABLE 1. *H. influenzae strains used for inoculation of blood culture media*

Strain of *H. influenzae*	Inoculum[a]
Capsular types	
a	14
b	12
c	16
d	15
Nontypables	
1	17
2	14
3	15
4	10

[a] Inoculum expressed as number of viable organisms per bottle. The standard error associated with each inoculum was 1.3.

Preparation of human serum and erythrocytes. A single lot of pooled human serum was used in this investigation. Twenty healthy volunteers who were not on antibiotic therapy during the preceding 2 weeks served as donors of blood. The serum was separated by centrifugation at 2,000 rpm for 15 min, sterilized by filtration (0.22-μm pore size; Millipore Corp.), and stored at −70°C until use to prevent the inactivation of complement. Group "O" erythrocytes were obtained from the hospital blood bank approximately 2 days after donation and washed three times with saline. The serum and erythrocytes were tested for sterility.

Inoculation and evaluation of blood culture media. The rubber stopper of each blood culture bottle was cleansed with 70% alcohol, disinfected with Betadine for 1 min, and washed with 70% alcohol. One milliliter of BHI broth containing test microorganisms (Table 1) was injected into duplicate blood culture bottles with or without RBC-SM. The RBC-SM contained 2.25 ml of erythrocytes and 2.75 ml of serum. The bottles were initially agitated, incubated unvented at 35°C, and subcultured 1, 4, 7, and 14 days after inoculation. Subculturing was performed by inoculating chocolate agar plates with 0.2 ml of blood culture medium. Plates were incubated for 24 h at 35°C in an environment containing 5% CO_2 and 95% air. Recovery of 87% (seven of the eight strains) or greater of the test microorganisms from the blood culture media within 4 days after inoculation was considered to be a satisfactory recovery rate.

RESULTS

Duplicate blood culture media as obtained from the manufacturers were inoculated with individual strains of *H. influenzae* (Table 1). The cumulative percentages of the microorganisms recovered from the blood culture media without supplementation with the RBC-SM are shown in Table 2. Twenty-four hours after inoculation, BHI broth from GIBCO supported the growth of 63% (five of eight) of the strains of *H. influenzae*. The majority (22 of 23) of the blood culture media supported the growth of 50% or less of the test microorganisms. In fact, no growth of *H. influenzae* was detected from 7 of the 23 blood culture media. Improved recovery rates of the *H. influenzae* strains were detected in the majority of the blood culture media 4, 7, or 14 days after inoculation. BHI and tryptic soy (TS) broths from GIBCO, however, were the only blood culture media that supported the growth of 87% of the strains of *H. influenzae* within 4 days after inoculation. Seven days after inoculation, Columbia broth (GIBCO) supported the growth of 87% of the strains.

TABLE 2. *Cumulative percentage[a] of H. influenzae strains recovered from different types of blood culture media*

Blood culture medium[b] (commercial source)	Days after inoculation			
	1	4	7	14
BHI (BBL)	13	25	50	63
BHI (Difco)	37	50	63	75
BHI (GIBCO)	63	87	87	87
BHI (Pfizer)	13	25	25	63
Brucella (Pfizer)	0	0	13	37
Brucella + sucrose (Pfizer)	0	13	13	25
Columbia (BBL)	13	25	25	37
Columbia (BD)	13	25	37	50
Columbia (Difco)	13	25	37	63
Columbia (GIBCO)	50	75	87	87
Columbia + 10% sucrose (GIBCO)	13	13	25	25
Columbia + 20% sucrose (GIBCO)	0	13	25	25
Columbia (Pfizer)	0	25	37	37
Columbia (Scott)	25	37	37	37
Dextrose phosphate (GIBCO)	25	50	50	63
Lederle	0	25	25	25
Supplemented peptone (BD)	25	63	63	75
Thiol (Difco)	13	25	25	25
TS (BBL)	0	0	25	50
Trypticase Soy (BD)	13	13	25	50
TS (Difco)	50	63	75	75
TS (GIBCO)	50	87	87	87
TS (Pfizer)	0	13	13	25

[a] Percentage represents the number of microorganisms obtained from duplicate blood culture media.

[b] Media contained sodium polyanethol sulfonate, except for Columbia and Trypticase soy broths of Becton, Dickinson (BD), which contained sodium amylosulfate. The concentration of supplement ranged from 0.025 to 0.05%.

In the second part of this investigation, blood culture media were supplemented with the RBC-SM and inoculated with individual suspensions of the strains of *H. influenzae* (Table 3). Incorporation of the RBC-SM into the blood culture media improved the recovery rates of the strains of *H. influenzae*. For example, 1 day after inoculation 60% (14 of 23) of the media supplemented with the RBC-SM showed improved recovery of the strains of *H. influenzae* (Table 3) when compared to blood culture media without the RBC-SM (Table 2). At the same time interval, however, no blood culture media recovered 87% or more of the strains of *H. influenzae*. A maximum recovery rate of 63% was

TABLE 3. *Cumulative percentage[a] of H. influenzae strains recovered from different types of blood culture media supplemented with RBC-SM*

Blood culture medium[b] (commercial source)	Days after inoculation			
	1	4	7	14
BHI (BBL)	13	25	50	50
BHI (Difco)	37	63	87	87
BHI (GIBCO)	63	87	100	100
BHI (Pfizer)	25	50	63	63
Brucella (Pfizer)	13	13	25	37
Brucella + sucrose (Pfizer)	13	25	25	37
Columbia (BBL)	25	63	63	75
Columbia (BD)	25	63	75	75
Columbia (Difco)	37	63	87	87
Columbia (GIBCO)	50	75	75	87
Columbia + 10% sucrose (GIBCO)	13	25	50	50
Columbia + 20% sucrose (GIBCO)	0	13	25	37
Columbia (Pfizer)	13	75	75	75
Columbia (Scott)	37	50	63	63
Dextrose phosphate (GIBCO)	25	63	75	87
Lederle	25	63	75	75
Supplemented peptone (BD)	50	87	100	100
Thiol (Difco)	25	37	50	50
TS (BBL)	13	25	37	63
Trypticase soy (BD)	25	50	63	75
TS (Difco)	37	50	87	87
TS (GIBCO)	25	87	100	100
TS (Pfizer)	13	37	50	50

[a] Percentage represents the number of microorganisms obtained from duplicate blood culture media.

[b] Media contained sodium polyanethol sulfonate, except for Columbia and Trypticase soy broths of Becton, Dickinson (BD), which contained sodium amylosulfate. The concentration of the supplement ranged form 0.025 to 0.05%.

obtained with GIBCO BHI broth. Four days after inoculation, BHI and TS broths from GIBCO and supplemented peptone broth from Becton, Dickinson and Co. supported the growth of 87% of the microorganisms. Seven days after inoculation, these three broths supported the growth of 100% of the strains of *H. influenzae*. An additional five blood culture media (Difco BHI, Columbia, and TS broths; GIBCO dextrose phosphate and Columbia broths) supported the growth of 87% of the microorganisms 7 or 14 days after inoculation.

DISCUSSION

The results of this investigation have demonstrated that the type of medium, its manufacturer, and the RBC-SM influence the ability of blood culture media to support the growth of *H. influenzae*. The majority of the blood culture media failed to support the growth of 87% or greater of the test microorganisms within 4 days after inoculation. In general, enhanced recovery of the strains of *H. influenzae* was obtained from BHI broth as compared to TS, Columbia, and the other types of blood culture media. Enhanced recovery of aerobic and facultatively anaerobic microorganisms from BHI broth has been reported (1, 5). This suggests that BHI blood culture medium provides an optimal environment for the growth of *H. influenzae* and other fastidious and facultative microorganisms.

Although BHI broth demonstrated an enhanced recovery of the strains of *H. influenzae*, a difference in the percent and the time of recovery was detected based upon the manufacturer of the medium (Table 2). For example, recovery of 87% of the microorganisms was obtained from BHI broth from GIBCO, whereas the BHI broths of BBL, Pfizer, and Difco supported the growth of only 25 to 50% of the strains of *H. influenzae* 4 days after inoculation. Similarly, TS broth from GIBCO supported the growth of 87% of the strains of *H. influenzae*; whereas the remaining sources of this medium supported the growth of 63% or less of the strains 4 days after inoculation (Table 2). These results demonstrate that the growth-promoting environment of the blood culture media is influenced by the manufacturer of the medium.

Incorporation of the RBC-SM into the blood culture media improved the recovery of the strains of *H. influenzae* (Table 3) as compared to media not supplemented with RBC-SM (Table 2). For example, 43% of the blood culture media supported the growth of *H. influenzae* type B in the absence of RBC-SM, whereas 69% of the blood culture media supported the growth of this strain after the addition of RBC-SM. Likewise, improved recovery of the other strains of *H. influenzae* was detected upon addition of RBC-SM to the blood culture media. The addition of RBC-SM, however, failed to alleviate the effect of the type of blood culture medium and the manufacturer on the growth of *H. influenzae*.

It would be important to know whether differences in strains influenced the recovery of *H. influenzae* from the blood culture media. Nontypable isolate no. 3 and capsular types C and D were the most frequent strains of *H. influenzae* that the blood culture media failed to support in the presence or absence of RBC-SM. In fact, 43% of the blood culture media containing RBC-SM failed to support the growth of these strains 14 days after inoculation. Although all strains of *H. influenzae* were recovered from the BHI and TS broths of GIBCO and the Becton, Dickinson supplemented peptone broth (Table 3), a delay in the recovery of these strains (C, D, and 3) was observed (4 to 7 days after inoculation). No difference in the time of detection of the remaining strains of *H. influenzae* (A, B, 1, 2, and 4) was observed. The ability of blood culture media to support the growth of *H. influenzae* was influenced by a difference in strain.

H. influenzae is one of the important etiolog-

ical agents of serious clinical infections in children (4, 10–12, 16) and has been shown to be increasing in adults (2, 3, 6, 9, 13, 14, 19, 24). Blood cultures have been considered an important part in the diagnosis of meningitis, epiglottitis, cellulitis, endocarditis, and septic arthritis (16, 20). The incidence of *H. influenzae* bacteremia in these diseases has been shown to depend upon host factors (13, 16, 19, 20) and blood collecting methods (3, 11). The results of this investigation also showed that the recovery of *H. influenzae* can be dependent upon the type of blood culture medium. Similarly, Hodge and Bremner (5) in a clinical evaluation of several blood culture media demonstrated that the type of medium influenced the early isolation of *H. influenzae* and *H. parainfluenzae*. Washington (21–23) and Rosner (15) also have shown that blood culture media differ in their ability to support the growth of *H. influenzae*. Since *H. influenzae* requires X (hemin) and V (nicotinamide adenine dinucleotide) factors for growth, the results of this investigation and those of Hodge and Bremner (5) suggest that BHI blood culture medium is sufficiently enriched to support the growth of *H. influenzae*. In addition to BHI broth, this investigation demonstrated that TS broth from GIBCO and supplemented peptone broth from Becton, Dickinson were also superior to the remaining types of blood culture media for recovery of strains of *H. influenzae*.

The conclusions drawn from this investigation are based upon conditions that have attempted to simulate clinical specimens. The selection of a blood culture medium based on results presented in this investigation should be exercised with caution, since a clinical evaluation is necessary. The results of this investigation, however, confirm our previous observations (1) that certain blood culture media need to be improved. Since the recovery of *H. influenzae* from blood culture media was dependent upon the type and source of medium, it is apparent that better quality control measures are required to evaluate the growth-promoting environment of commercial blood culture media. A simulated system as described in this paper may be the method by which the manufacturer could evaluate the medium prior to shipment to clinical microbiology laboratories. Inoculation of blood culture media with well-characterized fastidious microorganisms supplied by a microbiology regulatory agency might increase and insure the quality of commercial blood media.

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