International Journal Dental and Medical Sciences Research (IJDMSR)
ISSN: 2393-073X Volume 2, Issue 10 (Oct- 2018), PP 55-59
www.ijdmsr.com

Detection and Evaluation of Bacteremia in Twenty Different Blood Culture Media - a comparative evolution

Dr. Prakash Chandra Jha[1]
Senior Resident, Department of Oral Medicine and Radiology, Patna Dental College and Hospital, Patna 4.
**Corresponding Author: Dr. Prakash Chandra Jha*

ABSTRACT:- Several investigators have evaluated clinically a variety of commercially available blood culture media. No agreement has been reached as to which of these media is optimal for detection of bacteremia. The purpose of this study was to determine the rate of recovery of microorganisms from various blood culture media. A total of 20 blood culture media were inoculated with 7 to 15 microorganisms per bottle in the presence or absence of an erythrocyte-serum mixture. The results demonstrated that blood culture media differed in their ability to support the growth of microorganisms. At 4 days after inoculation, only 10 of the 20 blood culture media supported the growth of 91% (10 of the 11) or more of the test microorganisms. The recovery rate of microorganisms depended not only upon the type of medium but also upon the manufacturer of the type of blood culture medium. The addition of an erythrocyte-serum mixture to the blood culture media did not influence the difference in the recovery rate of microorganisms among media and the same type of médium prepared by different manufacturers. The majority (15 of the 20) of the blood culture media supplemented with the erythrocyte-serum mixture failed to support the growth of 91% or more of the test microorganisms at 4 days after inoculation. These results have demonstrated that blood culture media need to be improved. Better quality control measures should also be implemented to evaluate commercial blood culture media.

I. INTRODUCTION

The prompt and accurate isolation of the etiological agent of bacteremia is one of the most important functions performed by clinical microbiology laboratories. It is important that the infecting organism be detected and identified rapidly to guide proper therapy. One of the major variables affecting the isolation of bacteremic agents is the blood culture medium. Several investigators (1-3, 6, 7, 11, 12, 16, 17, 19, 20) have evaluated clinically the blood culture media to determine the optimal medium for isolation of microorganisms. Blood culture media were inoculated in parallel with the blood of patients and the recovery rates of the bacteremic agents were compared. Unfortunately, no agreement has been reached as to which of the commercially available blood culture media is optimal for the isolation of a wide variety of microorganisms (5-7, 10, 13-15, 17, 21, 22). It is estimated that there are over 200 separate blood culture media available today. The parallel clinical evaluation of these multiple blood culture media would require a large volume of blood from a bacteremic individual and possibly lead to medical complications. To circumvent any clinical complication, we chose to evaluate multiple blood culture media by using simulated clinical specimens. A total of 20 blood culture media were inoculated with a suspension of individual microorganisms with or without an erythrocyte-serum mixture (RBC-SM). The recovery rates of microorganisms from individual blood culture media were compared.

II. MATERIAL AND METHODS

Blood culture media. A total of 20 blood culture media were purchased from seven commercial manufacturers. Blood culture bottles contained approximately 50 ml of medium supplemented with either sodium polyanetholesulfonate or sodium amyl sulfate. The types and commercial sources of the blood culture media are listed in Table 1. Microorganisms. The microorganisms prepared for use as stock cultures in this investigation are listed in Table 2. These microorganisms were chosen to represent those frequently isolated from bacteremic individuals or having fastidious growth requirements. Seed cultures were prepared by inoculating brain heart infusion (BHI) broth with a single colony obtained from a recent clinical isolate. Separate 100-ml samples of BHI broth were inoculated with individual seed cultures. A coenzyme-vitamin-amino acid enrichment (Grand Island Biological Co. [Gibco]) was added to BHI broth to facilitate the growth of microorganisms. After incubation at 35°C for 18 h, the cultures were 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 microorganism were placed in vials, sealed, and stored in liquid nitrogen.

Determination of the number of microorganisms used for inoculation of blood culture media.

Frozen vials containing the microorganisms were thawed, and the bacterial suspensions were serially diluted to yield a final concentration of 7 to 15 microorganisms per ml (Table 2). This inoculum was chosen to simulate a low grade bacteremia (4, 8, 9). To determine the actual number of microorganisms introduced into each blood culture medium, a 0.5-ml sample of the inoculum was plated on chocolate agar plates. Colonies were counted after a 24-h incubation at 35°C, and the number of microorganisms per milliliter of inoculum was determined.

Preparation of human serum and erythrocytes. A single lot of pooled human serum was used in this investigation. 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-,um 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 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 Surgical Scrub/Skin Cleanser for 1 min, and washed with 70% alcohol. A 1-ml quantity of BHI broth containing test microorganisms (Table 2) was injected into duplicate blood culture bottles with or without RBC-SM. The RBC-SM contained 2.75 ml of erythrocytes and 2.25 ml of serum. The bottles were agitated, incubated unvented at 35°C, and subcultured at 1, 4, 7, and 14 days after inoculation. Subculturing was performed by inoculating chocolate agar plates with 0.1 to 0.2 ml of blood culture medium. Recovery of 91% (10 of 11) or more of the test microorganisms from the blood culture media within 4 days after inoculation was considered to be a satisfactory recovery rate.

Commercial source	Type of blood culture medium'
BBL	BHI broth
BBL	Columbia broth
BBL	Trypticase soy broth
BD	Columbia broth
BD	Supplemented peptone broth
BD	Trypticase soy broth
Difco	BHI broth
Difco	Columbia broth
Difco	Thiol broth
Difco	TSB
Gibco	BHI broth
Gibco	Columbia broth
Gibco	Columbia broth + 10% sucrose
Gibco	Columbia broth + 20% sucrose
Gibco	Dextrose phosphate broth
Gibco	TSB
Lederle	Lederle blood culture media
Pfizer	BHI broth
Pfizer	Brucella broth
Pfizer	Brucella broth + 10% sucrose

TABLE 1. Commercial blood culture media inoculated with aerobic and facultative anaerobic microorganisms

Acinetobacter calcoaceticus subsp. lwoffi	13
Candida albicans	7
Escherichia coli	15
Haemophilus influenza type b ..	12
Klebsiella pneumoniae	14
Neisseria meningitidis	14
Pseudomonas aeruginosa	12
Staphylococcus aureus	8
Streptococcus faecalis	10
Streptococcus pneumoniae	14

TABLE 2. Aerobic and facultative anaerobic microorganisms used for inoculation of blood culture media

III. RESULTS

Evaluation of blood culture media. The purpose of this experiment was to determine the ability of 20 blood culture media (Table 1) as obtained from the manufacturers to support the growth of 11 test microorganisms (Table 2). Duplicate blood culture media were inoculated with individual suspensions of microorganisms, incubated, and subcultured. The percentages of microorganisms recovered from the blood culture media are shown. Peptone broth from Becton, Dickinson & Co. (BD) supported the growth of 100% of the test microorganisms within 24 h after inoculation. At the same time interval, an additional six blood culture media supported the growth of 91% of the test microorganisms. These results demonstrated that only 7 of the 20 blood culture media supported the growth of 91% or more of the test microorga-nisms within 24 h after inoculation. At 4 days after inoculation, BHI broths from Baltimore Biological Laboratory (BBL), Difco Laboratories, and Gibco, Columbia broth from BBL, and tryptic soy broth (TSB) from Gibco supported the growth of 100% of the test microorganisms. In addition, Columbia broths from BD and Gibco, Scott Laboratories, Inc., and Lederle blood culture medium from American Cyanamid Co., Lederle Laboratories Div. supported the growth of 91% of the test microorganisms. Haemophilus influenza, Neisseria meningitidis, and Streptococcus pneumoniae were the most frequent microorganisms that the blood culture media failed to support. The most fastidious microorganism, H. influenza, was isolated in 24 h from only 1 of the 20 blood culture media. At 4 days after inoculation, four additional blood culture media supported the growth of H. influenzae. The less fastidious microorganisms' N. meningitidis and S. pneumoniae were recovered from six and nine of the blood culture media at 1 and 4 days after inoculation, respectively. Nonfastidious microorganisms, such as Escherichia coli, Klebsiella pneumoniae, and Streptococcus faecalis, were recovered from 100% of the blood culture media within 4 days after inoculation. Evaluation of blood culture media supplemented with RBC-SM. A total of 20 blood culture media (Table 1) were supplemented with RBC-SM, inoculated with individual suspensions of test microorganisms, incubated, and subcultured. BHI broth from Gibco supported the growth of 100% of the test microorganisms at 24 h after inoculation. At the same time interval, BHI broths from Difco and Pfizer Inc., Columbia broth from Gibco, and TSB from Difco supported the growth of 91% of the test microorganisms. At 4 days after inoculation, 100% recovery of the microorganisms was obtained from eight additional blood culture media (Table 4). Supplementation of the blood culture media with RBC-SM improved the recovery of H. influenza when compared with media not supplemented with RBC-SM. Improved isolation of H. influenza was detected in BHI broths from Gibco and Pfizer, Columbia broths from Gibco and Scott, Dextrose phosphate broth from Gibco, and TSB from Difco. The RBC-SM also improved the recovery of N. meningitidis and S. pneumoniae, but no statistically significant improvement was detected in the isolation rates of less fastidious microorganisms, such as E. coli, K. pneumoniae, and S. faecalis. These results suggested that the RBCSM played an important role in the recovery of fastidious microorganisms from some blood culture media. The majority of the blood culture media (15 of the 20) showed either no change or a decrease in the recovery of microorganisms upon addition of RBC-SM.

IV. DISCUSSION

Selection of an optimal blood culture medium for the rapid detection of the etiological agent of bacteremia is one of the most important decisions made by clinical microbiologists. Frequently, a clinical microbiologist selects a blood culture medium based upon clinical studies that have evaluated multiple blood culture media in parallel (3, 5, 6, 14, 16-18, 20) or upon information supplied by the manufacturers of the blood culture media. A review of the literature (4, 7, 10, 15, 17, 19, 22) has shown that no consensus exists as to which of the available blood culture media is the optimal system for recovery of a wide spectrum of aerobic and facultatively anaerobic microorganisms. A number of variables, such as variety of blood culture media, density of bacteria in the blood of a patient, time of blood collection, and immune response of the host have contributed

to the confusion as to which of the available commercial (6, 16, 17, 20) and "homemade" (12) blood culture media is the optimal one for rapid detection of bacteremia.

We have demonstrated that the type of medium, the manufacturer, and the RBC-SM have an effect on the ability of blood culture media to support the growth of microorganisms. Evaluation of 20 blood culture media with or without RBC-SM demonstrated that the majority of the media failed to support the growth of 91% (10 of 11) or more of the test microorganisms within 4 days after inoculation. Table 3 shows that the blood culture media differed in their ability to support the growth of fastidious aerobic and facultatively anaerobic microorganisms. In general,

BHI broth showed enhanced isolation of fastidious and nonfastidious microorganisms when compared with TSB, Columbia broth, and the other blood culture media. This suggested that BHI broth contained nutritional factors that enhanced the recovery of microorganisms or contained fewer microbial inhibitors than did the other media. The recovery of microorganisms from blood culture media not supplemented with RBC-SM was also dependent upon the manufacturer of the type of medium. Although BHI broth showed an enhanced recovery of microorganisms when compared with the other types of media, a slight difference in the percentage and time of recovery was detected based upon the manufacturer of this type of medium. BHI broths from BBL and Difco supported the growth of 100% of the test microorganisms within 4 days after inoculation, whereas 91% or less recovery of microorganisms was obtained from BHI broths from Gibco and Pfizer within 4 days after inoculation. The differences in recovery rates of microorganisms were more pronounced when the other types of commercially prepared media were compared. Columbia broths from Gibco and BBL supported the growth of 91% of the test microorganisms, whereas Columbia broths prepared by Difco, Pfizer, BD, and Scott recovered less than 50% of the test microorganisms in 24 h. Similarly, TSB from Gibco and Pfizer supported the growth of 81% or more of the test microorganisms, whereas TSB prepared by Difco, BBL, and BD recovered only 45% of the microorganisms in 24 h. These results emphasized that blood culture media prepared by different manufacturers influenced the recovery rate of microorganisms. Inoculation of blood culture media with RBCSM failed to alleviate the difference in recovery rates of microorganisms among the types of media and the same type of medium prepared by different manufacturers. In fact, incorporation of RBC-SM into the blood culture media failed to improve the recovery rates of microorganisms from the majority (15 of the 20) of the blood culture media when compared with blood culture media not supplemented with RBC-SM.

Rapid detection of the etiological agent of bacteremia is extremely important. Evaluation of the 20 blood culture media supplemented with RBC-SM showed that BHI broths from Difco, Gibco, and Pfizer, Columbia broths from Gibco and Scott, TSB from Difco and Gibco, and Dextrose phosphate broth from Gibco supported the growth of 100% of the test microorganisms within 4 days after inoculation. BHI broth from Gibco was the only blood culture medium that supported the growth of 100% of the test microorganisms within 24 h after inoculation. Caution must be exercised in interpreting these results as recommendations for the selection of an optimal blood culture medium until additional clinical studies have been performed.

V. CONCLUSION

The results of this investigation clearly demonstrate that blood culture media need to be improved. To support the growth of fastidious microorganisms, the nutritional capacity of certain blood culture media should be augmented, or the inhibitory substances should be removed. It is apparent that better quality control measures are needed to evaluate the growth-promoting ability of commercial blood culture media by clinical microbiologists and the manufacturers.

Quality control of the blood culture media is absolutely necessary, because there is a difference in recovery rates of microorganisms from the same medium prepared by different manufacturers.

Inoculation of blood culture media with microorganisms should be an integral part of the quality control program to evaluate commercial and homemade blood culture media.

REFERENCES

[1]. Coetzee, E. F. C., and R. S. A. Johnson. 1974. An improved blood culture medium. Med. Lab. Technol. 31:299-303.

[2]. Conner, V., and O. T. Mallery, Jr. 1951. Blood culture: a clinical laboratory study of two methods. Am. J. Clin. Pathol. 21:785-788.

[3]. Dalton, H. P., R. Krammer, M. R. Escobar, and M. J. Allison. 1974. Comparison of two blood culture systems. Am. J. Clin. Pathol. 61:856-858.

[4]. Finegold, S. M., M. L. White, I. Ziment, and W. R. Winn. 1969. Rapid diagnosis of bacteremia. Apple. Microbiol. 18:458-463.

[5]. Gantz, N. M., J. L Swain, and A. A. Mediros. 1975. Vacuum blood culture bottles: inhibiting growth of Candida and favoring growth of Bacteroides. Lancet ii:1174-1176.

[6]. Gross, P. A., R. Fryda, and K. Reilly. 1977. Comparative evaluation of different types of blood culture media for isolation of aerobes. J. Clin. Microbiol. 6:362-366.
[7]. Hall, M., E. Warren, and J. A. Washington II. 1974. Comparison of two liquid blood culture media containing sodium polyanetholesulfonate: tryptic soy and Columbia. Apple. Microbiol. 27:699-702.
[8]. Le Frock, J. L., C. A. Ellis, J. B. Turchik, J. K. Zawacki, and L. Weinstein. 1975. Transient bacteremia associated with percutaneous liver biopsy. J. Infect. Dis. 131:S104-S107.
[9]. Le Frock, J. L., A. S. Klainer, W. H. Wu, and H. Turndorf. 1976. Transient bacteremia associated with nasotracheal suctioning. J. Am. Med. Assoc. 231: 1267-1269.
[10]. Morello, J. A., and P. D. Ellner. 1969. New medium for blood cultures. Apple. Microbiol. 17:68-70.
[11]. Painter, B. G., and H. D. Isenberg. 1975. Clinical laboratory evaluation of the fifty-milliiter vacutainer blood culture tube. J. Clin. Microbiol. 2:99-103.
[12]. Qadri, S. M. H., and R. D. Wende. 1977. A single-bottle blood culture system: evaluation and comparison with two other systems. Can. J. Microbiol. 23:1041-1044.
[13]. Roberts, G. D., C. Horstmeier, M. Hall, and J. A. Washington II. 1975. Recovery of yeast from vented blood culture bottles. J. Clin. Microbiol. 2:18-20.
[14]. Rosner, R. 1968. Effect of various anticoagulants and no anticoagulants on ability to isolate bacteria directly from parallel clinical blood specimens. Am. J. Clin. Pathol. 49:216-219.
[15]. Rosner, R. 1970. Comparison of a blood culture system containing liquoid and sucrose with systems containing either reagent alone. Apple. Microbiol. 19:281-282.
[16]. Rosner, R. 1972. A quantitative evaluation of three blood culture systems. Am. J. Clin. Pathol. 57:220-227.
[17]. Rosner, R. 1974. Evaluation of four blood culture systems using parallel culture methods. Apple. Microbiol. 28:245-247.
[18]. Sullivan, N. M., V. L. Sutter, W. T. Carter, H. R. Attebery, and S. M. Finegold. 1972. Bacteremia after genitourinary tract manipulation: bacteriological aspects and evaluation of various blood culture systems. Appl. Microbiol. 23:1101-1106.
[19]. Washington, J. A. Il. 1971. Comparison of two commercially available media for detection of bacteremia. Appl. Microbiol. 22:604-607.
[20]. Washington, J. A. Il. 1972. Evaluation of two commercially available media for detection of bacteremia. Appl. Microbiol. 23:956-959.
[21]. Washington, J. A. II. 1975. Blood cultures: principles and techniques. Mayo Clin. Proc. 50:91-98.
[22]. Washington, J. A. HI, M. M. Hall, and E. Warren. 1975. Evaluation of blood culture media supplemented with sucrose or with cysteine. J. Clin. Microbiol. 1:79-81.

Corresponding Author: Dr. Prakash Chandra Jha
Senior Resident, Department of Oral Medicine and Radiology,
Patna Dental College and Hospital, Patna 4.