e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15 (9); 1414-1419

Original Research Article

Comparison of Efficacy of Biphasic BHI Media, Tryptic Soy Broth and Glucose Broth in Isolating Bacteria through Blood Culture of Suspected Septicemia Patients

Ruchi Agrawal[1], Mitesh Shah[2], Puja Singh[3], K.P. Ranjan[4]

[1]Demonstrator, Department of Microbiology, Bundelkhand Medical College, Sagar, (M.P.)
[2]Assistant Professor, Department of Pathology, Bundelkhand Medical College, Sagar, (M.P.)
[3]Associate Professor, Department of Pathology, Bundelkhand Medical College, Sagar, (M.P.)
[4]Associate Professor, Department of Microbiology, Gajra Raja Medical College, Gwalior, (M.P.)

Received: 25-06-2023 / Revised: 28-07-2023 / Accepted: 30-08-2023
Corresponding author: Dr. Mitesh Shah
Conflict of interest: Nil

Abstract:

Introduction: Blood stream infection (BSI), bacteremia and thereby septicaemia remains one of the most important causes of morbidity and mortality throughout the world. The diagnosis of these infections can be confirmed by blood culture which is the gold standard for the diagnosis. No single blood culture medium or system is capable of detecting all the micro-organisms. Present study compares the efficacy of three culture media for isolation of bacteria through blood culture of suspected septicemia patients.

Material & Methods: Study was conducted in Department of Microbiology, Gajra Raja Medical College, Gwalior (M.P.) over suspected septicemia patients for a period of one year. A total of 367 samples of blood culture used in the study. Blood was transferred to three blood culture bottles containing three different media as Biphasic BHI media, Tryptic Soy broth and Glucose broth then processed for isolation of bacteria.

Result: Out of total 367 suspected septicemia patients, bacteria were isolated in 98 samples. Out of 98 culture positive cases, 62 isolates were gram negative bacilli and 36 isolates were gram positive cocci. Among gram-positive bacteria biphasic BHI bottle recovered significantly more Staphylococcus aureus bacteria ($P < 0.037$) than do the TSB bottle or Glucose broth bottle while for rest of bacteria no statistically significant difference was found among three medias.

Conclusion: As present study show superiority of Biphasic BHI over TSB and glucose broth in isolating Staphylococcus aureus only. So all the three culture media seems to have almost similar efficacy in isolating organisms in septicemia patients.

Keywords: Septicemia, Biphasic BHI media, Tryptic Soy broth and Glucose broth.

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.

Introduction

Septicemia is presence of microbes or its toxins in blood while bacteremia is presence of bacteria in blood [1]. Septicemia may be a transient, self-limited phenomenon without clinical consequences or with clinical manifestation and systemic response that can lead to multiorgan failure and finally to death. It frequently reflects the presence of serious infection [2]. It is often associated with hospitalization, insertion of foreign bodies such as catheters into blood vessels, and other predisposing factors like ICU, lapses in hand washing, and non-adherence to infection control practices of medical staff [3]. Genitourinary tract, intraabdominal foci and respiratory tract are the common sources of blood stream infections [4,5].

Blood stream infection (BSI), bacteremia and thereby septicaemia remains one of the most important causes of morbidity and mortality throughout the world. Annually about 200,000 cases of bacteremia occur worldwide with mortality rates of 20-50% approximately. BSI accounts for 10-20% of all health care associated infections and is the eighth leading cause of mortality. It is a significant health problem in developing countries [6].

The diagnosis of these infections can be confirmed by blood culture which is the gold standard for the diagnosis, and is routinely available in hospitals of developing countries like India [7,8]. A suspected sepsis episode was classified as proven when a definite pathogen was isolated from a blood culture, or a possible pathogen was isolated in the presence of a central vascular catheter or from two blood samples taken from two different puncture sites [9]. In the study of **Gupta S. et al.** out of total 3472 blood samples 16.5% of samples yielded

growth. Among positive blood culture samples, 96.68% yielded bacterial isolates and 3.31% were Candida species. Gram-positive and gram-negative organisms contributed to 41.65% and 58.34% respectively. E. coli was the predominant organism isolated (22.4%) followed by Klebsiella spp. (19.7%) and S. aureus (18.3%) respectively. E. coli (38.46%) was the most commonly isolated gram-negative bacteria followed by Klebsiella spp. (33.84%), Pseudomonas spp. (14.46%), Acinetobacter spp. (8.92%) and Salmonella typhi (1.53%) of total gram-negative isolates from blood culture. The predominant isolate among the gram-positive isolates was Staphylococcus aureus (43.9%) followed by coagulase-negative staphylococci (41.8%) and Enterococcus spp. (11.6%) respectively [10].

In spite of newer molecular techniques, blood culture is still considered to be the 'gold standard' for the detection of microbial pathogens in bacteremia and sepsis [11]. Various commercial blood culture systems are available. They compete with regard to sensitivity for organism recovery, time to positivity, workload capacity, user interface and associated costs. No blood culture system is perfect and able to detect all the micro-organisms. All blood culture systems require inoculation of blood into a media bottle. Several studies have shown that the lysis centrifugation systems are more sensitive for the detection of fastidious organisms and dimorphic fungi. The continuous monitoring systems have shown superiority with regard to time to positivity compared to manual systems and currently are the preferred systems. Workload, user friendly, cost of implementation and maintenance may play important role in selecting blood culture system [11].

No single blood culture medium or system is capable of detecting all the micro-organisms. Many manufacturers supplement their base media with proprietary additives designed to enhance microbial growth, and it cannot be assumed that common generic media (e.g., supplemented soybean casein digest broth) from different manufacturers will perform in an equivalent manner [12]. Various culturing media within one system differ with regard to constituents and performance and the choice relies heavily on controlled clinical evaluation.

The media are similar in principle; however controlled clinical trials have shown some media to be superior for certain organisms. Sensitivity for organism recovery is the most import parameter when selecting a blood culture system [11]. In routine circumstances, blood cultures need not be incubated for >7 days. Incubation period longer than 7 days may be useful when fungemia or bacteremia due to fastidious organisms such as the HACEK group of bacteria or species of Legionella or Brucella are suspected. Mycobacterial blood cultures should be incubated for ~4 weeks [12].

Present study compares the efficacy of Biphasic BHI media, Tryptic Soy broth and Glucose broth for isolation of bacteria through blood culture of suspected septicemia patients.

Aims & Objectives

To isolate the bacteria causing septicemia in the patients and to correlate the Biphasic BHI media, Tryptic Soy broth and Glucose broth in terms of early growth of bacteria, used for blood culture.

Material & Methods:

Study was conducted in Department of Microbiology, Gajra Raja Medical College and J.A. group of hospitals, Gwalior (Madhya Pradesh) over suspected septicemia patients admitted in various wards for a period of one year from April 2017 to March 2018. A total of 367 samples of blood culture used in the study. Patients in Shock or any other co-morbid condition, not willing to participate, age group below 14 years and outdoor patients were excluded from study. Blood from suspected septicemia patients was collected transferred to three blood culture bottles containing three different media as Biphasic BHI media, Tryptic Soy broth and Glucose broth in the dilution of 1:10. Labelled bottles were transported along with requisition form to bacteriology section of department of Microbiology, Gajra Raja Medical College with minimal delay (15 minutes). All blood cultures were processed in the laboratory using standard procedure by conventional method. Blood culture bottles were incubated overnight at 37˚C then sub-cultured onto Blood agar and Mac-Conkey agar to look for growth. From the obtained growth isolated colonies were used for gram's staining and biochemical tests for the differentiation of organism by using the standard method. Blood culture bottle which shows no sign of growth was further incubated at 37˚C . Samples were reported as no growth only after 7 days of incubation. The statistical analysis was performed using standard tests. The data was represented as percentages and proportions. When two or more set of variables were compared Fisher's exact test was applied. If the p-value was <0.05, it was considered significant.

Result:

Out of total 367 suspected septicemia patients, bacteria were isolated in 98 samples. Out of the total 98 culture positive cases, 62 isolates (63.27%) were gram negative bacilli and 36 isolates (36.73%) were gram positive cocci.

Table 1: Distribution of bacteria in total 98 isolates of septicemia patients

S. No.		Bacterial Isolates	Number of isolates	Percentage
1	Gram negative bacteria (Fermenters)	Escherichia coli	14	14.29%
2		Klebsiella pneumoniae	12	12.24%
3		Klebsiella oxytoca	08	08.16%
4		Citrobacter koseri	05	05.10%
5		Citrobacter freundii	02	02.04%
6		Enterobacter aerogenes	04	04.08%
7	Gram negative bacteria (Non-fermenters)	Pseudomonas aeruginosa	06	06.12%
8		Acinetobacter baumannii	09	09.18%
9		Acinetobacter lwoffii	02	02.04%
10	Gram positive bacteria	Staphylococcus aureus	25	25.51%
11		Coagulase negative staphylococcus	11	11.22%

Table 1 shows that among the gram negative bacteria (fermenters group) Klebsiella spp. 20.41% (Klebsiella pneumonia-12.24% and Klebsiella oxytoca-08.16%) was most common isolate and in non-fermenters group Acinetobacter spp. 11.22% (A. baumannii 09.18 % and A. lwoffii 02.04%) was most commonly isolated. Among gram positive bacteria Staphylococcus aureus (25.51%) was the predominant isolate. Overall in present study predominant isolate was Staphylococcus aureus.

Table 2: Distribution of identified isolates in different blood culture media

Isolate	Biphasic BHI media	Tryptic Soy Broth	Glucose Broth
Escherichia coli (n= 14)	14	13	13
Klebsiella pneumonia (n= 12)	12	11	11
Klebsiella oxytoca (n= 08)	07	08	07
Citrobacter koseri (n=05)	04	05	05
Citrobacter freundii (n=02)	02	01	01
Enterobacter aerogenes (n=04)	04	04	03
Pseudomonas aeruginosa (n=06)	04	06	04
Acinetobacter baumanii (n=09)	09	08	08
Acinetobacter lwoffii (n=02)	01	02	01
Staphylococcus aureus (n=25)	25	21	22
CONS (n=11)	11	11	11
Total (n=98)	93	90	86

Table 2 shows that of the total 98 isolates, maximum number of isolates i.e. 93 was recovered from biphasic BHI bottle followed by 90 isolates from TSB and minimum isolates 86 from Glucose broth. No statistically significant differences between these three blood culture media in the number of identified isolates.

Comparison of identified isolates in different blood culture media

Table 3(a): Comparison between Biphasic BHI and TSB

Isolate	Biphasic BHI & TSB both	Biphasic BHI only	Tryptic Soy broth only
Escherichia coli (n= 14)	13	01	00
Klebsiella pneumonia (n= 12)	11	01	00
Klebsiella oxytoca (n= 08)	07	00	01
Citrobacter koseri (n=05)	04	00	01
Citrobacter freundii (n=02)	01	01	00
Enterobacter aerogenes (n=04)	04	00	00
Pseudomonas aeruginosa (n=06)	04	00	02
Acinetobacter baumanii (n=09	08	01	00
Acinetobacter lwoffii (n=02)	01	00	01
Staphylococcus aureus (n=25)	21	04	00
CONS (n=11)	11	00	00

Table 3 (a) shows the distribution of the isolates grown in Biphasic BHI and TSB broth bottles. Staphylococcus aureus was isolated significantly ($P < 0.037$) more frequently from the biphasic BHI bottle. Pseudomonas aeruginosa was isolated more frequently from the TSB bottle, but not statistically significant. There were no statistically significant differences between both bottles in the number of positive cultures by other bacteria.

Table 3(b): Comparison between Biphasic BHI and Glucose broth

Isolate	Biphasic BHI & Glucose broth both	Biphasic BHI Only	Glucose broth only
Escherichia coli (n= 14)	13	01	00
Klebsiella pneumonia (n= 12)	11	01	00
Klebsiella oxytoca (n= 08)	07	00	00
Citrobacter koseri (n=05)	04	00	01
Citrobacter freundii (n=02)	01	01	00
Enterobacter aerogenes (n=04)	03	01	00
Pseudomonas aeruginosa (n=06)	04	00	00
Acinetobacter baumanii (n=09)	08	01	00
Acinetobacter lwoffii (n=02)	01	00	00
Staphylococcus aureus (n=25)	22	03	00
CONS(n=11)	11	00	00

Table 3 (b) shows that Staphylococcus aureus was isolated significantly ($P < 0.037$) more frequently from the Biphasic BHI bottle and Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii, Enterobacter aerogenes, Acinetobacter baumanii were also isolated more frequently from the Biphasic BHI bottle, but non significantly. Citrobacter koseri was isolated more frequently from Glucose broth, but non significantly.

Table 3(c): Comparison between TSB and Glucose broth

Isolate	TSB & Glucose broth both	TSB only	Glucose broth only
Escherichia coli (n= 14)	13	00	00
Klebsiella pneumonia (n= 12)	11	00	00
Klebsiella oxytoca (n= 08)	07	01	00
Citrobacter koseri (n=05)	01	00	00
Citrobacter freundii (n=02)	05	00	00
Enterobacter aerogenes (n=04)	03	01	00
Pseudomonas aeruginosa (n=06)	04	02	00
Acinetobacter baumanii (n=09)	08	00	00
Acinetobacter lwoffii (n=02)	01	01	00
Staphylococcus aureus (n=25)	21	00	01
CONS (n=11)	11	00	00

Table 3 (c) shows that Klebsiella oxytoca, Enterobacter aerogenes, Pseudomonas aeruginosa and Acinetobacter lwoffii were isolated more frequently from the TSB while Staphylococcus aureus was isolated more frequently from Glucose broth, but there were no statistically significant differences in isolation of bacteria between the two culture media.

Table 4: Distribution of culture positivity in various media with respect to time of incubation

Blood culture media	24hrs	48hrs	72hrs	After 4 days	After 5 days	After 6 days	Total
Biphasic BHI broth	73	17	03	00	00	00	93
Tryptic Soy broth	72	16	02	00	00	00	90
Glucose broth	68	15	03	00	00	00	86

Table 4 shows that in all three blood culture media maximum cultures were positive by first subculture itself (after 24 hours of incubation) followed by second subculture (after 48 hrs) and third subculture (after 72 hrs) respectively, while virtually no isolates were obtained later (subcultured after 4, 5 and 6 days of incubation).

Table 5: Distribution of isolates in various media with respect to time of incubation

Time of incubation	Name of isolate	Biphasic BHI	TSB	Glucose broth
24 hrs	Escherichia coli	12	12	12
	Klebsiella pneumoniae	11	11	10
	Klebsiella oxytoca	07	07	06
	Citrobacter koseri	02	02	02
	Citrobacter freundii	02	01	01
	Enterobacter aerogenes	04	04	03
	Pseudomonas aeruginosa	02	02	01
	Acinetobacter baumannii	06	06	05
	Acinetobacter lwoffii	01	01	01
	Staphylococcus aureus	18	18	18
	Coagulase negative staphylococcus	08	08	09
48 hrs	Escherichia coli	02	01	01

	Klebsiella pneumonia	01	00	01
	Klebsiella oxytoca	00	01	01
	Citrobacter koseri	02	03	03
	Pseudomonas aeruginosa	02	03	02
	Acinetobacter baumannii	02	02	02
	Acinetobacter lwoffii	00	01	00
	Staphylococcus aureus	05	02	03
	Coagulase negative staphylococcus	03	03	02
72hrs	Pseudomonas aeruginosa	00	01	01
	Acinetobacter baumannii	01	00	01
	Staphylococcus aureus	02	01	01

Table5 shows that in all three media most of the bacteria were isolated after 48 hrs of incubation. Only P. aeruginosa (02 isolate), A. baumanii (01), S. aureus (01), were isolated after 72 hrs of incubation.

Discussion:

Comparison of three blood culture media

In the present study, analysis of the results was limited to the three blood culture bottles, i.e., biphasic BHI, TSB and Glucose broth. Total isolate was 98. Out of 98 isolates; the biphasic BHI recovered 93 (94.90%), the TSB recovered 90 (91.84%), and the Glucose broth were recovered 86 (87.76%) isolates.

Among gram-negative fermenter group number of recovered isolates were comparable in all three bottles, although among gram-negative non-fermenter group the TSB bottle recovered more isolates of Pseudomonas aeruginosa in comparison to other but not significantly. Among gram-positive bacteria the biphasic BHI bottle recovered significantly more Staphylococcus aureus bacteria ($P < 0.037$) than do the TSB bottle or Glucose broth bottle and CoNS were recovered same in all bottles.

In study by Hall et al [13] Staphylococcus aureus, was isolated significantly ($P < 0.01$) more from the Biphasic BHI bottle. Anaerobic bacteria, were isolated more frequently and significantly ($P < 0.01$) from the TSB bottle and Pseudomonas spp. too but none significantly. Study of Henry et al [14] showed comparison between the TSB and the Biphasic BHI bottles in the recovery of the 571 isolates. The TSB bottle isolated significantly more gram-negative bacteria particularly Enterobacteriaceae members. TSB bottle also isolated more Pseudomonas aeruginosa but non-significantly. Among gram-positive bacteria the Biphasic BHI isolated more Staphylococcus aureus and Staphylococcus epidermidis than TSB but not significantly while TSB isolated significantly more isolates of Streptococcus spp. than Biphasic BHI.

In the study done by Hall & Ilstrup et al [15] transiently vented TSB and BHI broth were compared, there were 264 isolates. No statistically significant differences between these two media were noted, either in numbers of isolates or in the average time required for their detection.

Time to detection

In present study Biphasic BHI showed 78.49% of the cultures were positive by first subculture itself (after 24 hours of incubation), 18.28% of the culture were positive by second subculture (after 48 hrs) and 03.23% of the culture were positive by third subculture i.e. after 72 hrs. In TSB 80.00%, 17.78%, 02.22% culture were positive after 24 hours, 48 hrs and 72 hrs respectively. In Glucose broth 79.07%, 17.44%, 03.49% culture were positive after 24 hours, 48 hrs and 72 hrs respectively. No isolate were obtained later (subcultured after 4, 5 and 6 days of incubation). Study done by Kante et al [16] observed that 32% of the cultures were positive by first subculture itself (after 24 hours of incubation of BHI broth), 43.4% and 17.65% of the culture were positive by second subculture (after 48 hrs) and third subculture (after 72 hrs) respectively, while only 05.88% isolates were obtained later (subcultured on day 7).

Conclusion

As present study show superiority of Biphasic BHI over TSB and glucose broth in isolating Staphylococcus aureus only. So all the three culture media seems to have almost similar efficacy in isolating organisms in septicemia patients. Though study with more number of samples to be conducted to assess efficacy of different media in isolating organisms from blood.

References

1. Fauci AS, Braunwald E. et al. Harrison Principle of Internal Medicine; 17th Edition, The McGraw Hill Companies, 2008; 1696.
2. Connie R. Mahon. Textbook of Diagnostic Microbiology. In: 5th edition. Bacteremia and Sepsis. Updated by Paula Mister, Donald C. Lehman. Elsevier, pg- 868-83.
3. Bhutta A, Gilliam C. et al. Reduction of bloodstream infections associated with catheters in paediatric intensive care unit: stepwise approach, BMJ 2007; 334: 362-5.

4. Seifert H, The Clinical Importance of Microbiological Findings in the Diagnosis and Management of Bloodstream Infections, Clinical Infectious Disease, s 2009; 48: S238–45.
5. Procop GW, Church DL. et al. Koneman's Color atlas and Textbook of Diagnostic Microbiology,7th Edition, Wolter Kluwer, 2016; 96-97.
6. Tak V, Mathur P. et al. Staphylococcal blood stream infections: Epidemiology, resistance pattern and outcome at a level 1 Indian trauma care center. J Lab Physicians. 2013; 5: 46–50.
7. Pal N, Sujatha R. Microbiological Profile and Antimicrobial Resistant Pattern of Blood Culture Isolates, Among Septicaemia Suspected Patients, National Journal of Laboratory Medicine, 2016; Vol 5(1): 17-21.
8. Pandey S, Raza S. et al. The Aetiology of the Bloodstream Infections in the Patients Who Presented to a Tertiary Care Teaching Hospital in Kathmandu, Nepal, Journal of Clinical and Diagnostic Research. 2013; 7(4): 638-641.
9. Guerti K, Devos H et al. Time to positivity of neonatal blood cultures: fast and furious? Journal of Medical Microbiology 2011, 60, 446–453
10. Gupta S, Kashyap B. Bacteriological profile and antibiogram of blood culture isolates from a tertiary care hospital of North India. Trop J Med Res, 2016; 19:94-9.
11. Dreyer AW. Blood Culture Systems: From Patient to Result, Sepsis – An Ongoing and Significant Challenge, InTech, 2012; Chapter 15: 287-310.
12. Weinstein MP. Current Blood Culture Methods and Systems: Clinical Concepts, Technology, and Interpretation of Results, Clinical Infectious Diseases, 1996; 23: 40-6.
13. Hall MM, Mueske CA. et al. Evaluation of a Biphasic Medium for Blood Cultures, Journal of Clinical Microbiology, 1979; 10(5):673-676.
14. Vandepitte J, Verhaegen J, Engbaek K, et al. Basic laboratory procedures in clinical bacteriology. 2ed. WHO: 2003
15. Hall MM, Ilstrup DM, Comparison of Three Blood Culture Media with Tryptic Soy Broth, Journal of Clinical Microbiology, 1978; 8(3): 299-301.
16. Kante M, Uma P. et al. Bacterial profile of blood stream infections and antibiotic susceptibility pattern of isolates, Int. J. Curr. Microbiol. App. Sci 2014; 3(12): 222-233.