ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 3, 2016

Online - 2455-3891
Print - 0974-2441

Short Communication

MODIFIED BIPHASIC MEDIA FOR BLOOD CULTURE

BHUVANESHWAR DEVARAJ, ARUNAVA KALI*, PRAVIN CHARLES MV, KUNIGAL SRINIVASAIAH SEETHA

Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Pondicherry - 607 402, India.
Email: ak.arunava@gmail.com

Received: 06 December 2015, Revised and Accepted: 29 February 2016

ABSTRACT

Objective: To evaluate the performance characteristics of Nutrient agar and MacConkey's agar biphasic medium (BPM) with conventional blood culture.

Methods: We prepared two modified biphasic blood culture medium with Nutrient agar (with 1% lactose and 0.004% neutral red) and MacConkey's agar. A total of 20 standard clinical strains of bacterial pathogens and *Candida albicans* were inoculated in duplicate in Nutrient agar BPM, MacConkey's agar, and brain heart infusion broth to evaluate the turn-around time and performance characteristics.

Results: All isolates except *Streptococcus pyogenes* and *Streptococcus pneumoniae* showed characteristic lactose fermenter and non-lactose fermenter colonies on Nutrient agar and MacConkey's agar BPM within 24 hrs with sufficient growth to carry out biochemical identification and antibiotic susceptibility testing. This resulted in a shorter turn-around time (48 hrs) when compared to that of conventional blood culture (72 hrs).

Conclusion: Nutrient agar and MacConkey's agar based modified BPM could be beneficial in reducing the turn-around time of blood culture.

Keywords: Biphasic media, Bloodstream infection, Conventional blood culture.

INTRODUCTION

Bloodstream infection is a serious medical condition. It is often associated with multidrug resistant pathogens causing potentially life-threatening infections involving other organ systems [1,2]. Diagnosis of bacteremia primarily depends on blood culture. Blood culture medium has a key role in isolating causative pathogens from blood. Several culture media for both conventional and automated blood culture systems are in use to facilitate rapid and optimum isolation of these pathogens. Biphasic blood culture medium (BPM), consisting broth and an agar slant, has a significant advantage over the conventional systems [3]. It precludes repeated open subcultures and thereby the risk of external contamination. The routinely used media for BPM slant are enriched media supporting the growth of fastidious organisms. Since these do not contain an indicator, lactose fermenter (LF), and lactose non-fermenter (NLF) colonies cannot be differentiated on BPM [4]. Fermentation of lactose is an essential characteristic which guides the selection of biochemical test panels for further identification [5]. In most laboratories, MacConkey's agar is routinely used for subculturing the microorganism from conventional blood culture systems for differentiating LF and NLF colonies. However, it increases the turn-around time to more than 18-24 hrs. Although the use of BPM is not uncommon, there are only a few studies which attempted incorporating the benefits of biphasic and differential media for blood culture. This study was carried out to determine the utility of two differential biphasic blood culture media in terms of performance characteristics in comparison to conventional blood culture.

We have utilized two sets of modified BPM and evaluated their performance characteristics with conventional blood culture, i.e., brain heart infusion (BHI) broth. Nutrient agar (with 1% lactose and 0.004% neutral red) and MacConkey's agar were used to prepare the BPM slants, whereas Nutrient broth was used as the blood culture broth in both sets. Vented blood culture bottles were used. Standard laboratory strains, i.e., *Staphylococcus aureus* (ATCC 25922 and 43300), *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus pneumoniae, Enterococcus faecalis, Escherichia coli* (ATCC 25923), *Klebsiella pneumoniae, Citrobacter freundii, Salmonella typhi, Salmonella paratyphi A, Shigella dysenteriae, Shigella flexneri, Shigella boydii, Shigella sonnei, Proteus mirabilis, Proteus vulgaris, Vibrio cholerae, Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii,* and *Candida albicans* were inoculated in duplicate in both Nutrient agar BPM, MacConkey's agar BPM, and BHI broth. A 2.5 ml inoculum of 0.5 McFarland turbidity was prepared in sterile normal saline from the overnight growth of these isolates on agar media, and it was injected into the blood culture media aseptically. The blood culture sets were incubated at 37°C aerobically, and daily subculture was made on the BPM slant by tilting the bottle and flooding the slant with blood culture broth. These were examined periodically for the growth of pathogen (turbidity in the broth and bacterial colonies on the slant).

We found that Nutrient agar and MacConkey's agar slants supported the growth of all isolates except *S. pyogenes* and *S. pneumoniae* and had no contamination. Although incorporation of neutral red indicator and lactose in Nutrient agar slants helped in accurate identification of most LF and NLF strains (Fig. 1a and b), we noted that strains of *Proteus* and *P. aeruginosa* developed a pale pink tinge, especially on prolonged incubation.

In contrast, the colony morphology of these strains was typical on MacConkey's agar slants. The bacterial isolates (except *S. pyogenes*

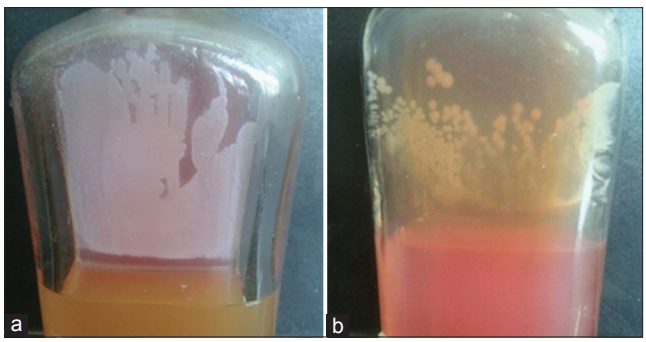

Fig. 1: (a and b) Lactose fermenter (left) and non-lactose fermenter (right) colonies on modified biphasic medium

and *S. pneumoniae*) produced visible growth of LF and NLF on BPM slants within first 24 hrs (48 hrs in case of *C. albicans*) facilitating rapid identification and antibiotic susceptibility testing with a turn-around time of 48 hrs. We compared it with the conventional blood culture. The total time taken to produce turbidity in the BHI broth, to recover bacterial colonies plating media, and to complete the identification with antibiotic susceptibility was 72 hrs on an average.

The success of blood culture reporting is limited by its long turn-around time. Although detectable growth in the blood culture broth permits initiation of antibiotics based on grams stain, targeted therapy with specific antibiotics require identification of the pathogen along with its antibiotic susceptibility. Isolation of the pathogen on plating media and identification of LF and NLF isolate on differential media like MacConkey's agar is an essential step for bacterial identification. Although automated blood culture systems have shorter turn-around time pertaining to continuous growth monitoring and early detection of positive cultures, they also require subculture on plating media. Furthermore, its affordability is limited to only a few centers. Various studies have utilized positive blood culture broth for direct identification and susceptibility testing to decrease the reporting time. However, the direct identification and susceptibility tests were often found unreliable which may be attributed to the low bacterial concentration in blood culture broth [6]. Use of blood culture BPM based on the differential medium is rare in literature. In a study conducted by Koshi *et al.*, the authors used MacConkey's agar BPM for blood culture and reported its advantages in reducing turn-around time [4]. In their study, MacConkey's agar BPM achieved good isolation of *Salmonella* and other Gram-negative bacilli. Furthermore, the growth on MacConkey's agar slant supported rapid identification of *Salmonella* by direct agglutination with specific antiserum. The majority of the blood culture isolates (67-82%) produced colonies on MacConkey's agar BPM slant within 48 hrs [4]. This is in accordance with our study. We used Nutrient agar with neutral red in addition to MacConkey's agar BPM in an attempt to obviate the inhibitory effect of MacConkey's agar on bile salt-sensitive bacteria, especially Gram-positive bacteria. However, no significant difference was observed. The limitation of this method is small sample size and inability to recover fastidious organisms and anaerobes. Further study with a large number of clinical isolates is essential to confirm these findings. This method is applicable for aerobic blood cultures. The aerobic non-fastidious organisms, which constitute the majority of blood culture isolates grew optimally and produced typical colonies on differential media used for BPM slant, allowing rapid identification and susceptibility testing. However, subculture may be necessary when isolated colonies are not available due to the presence of two or more organisms.

CONCLUSION

We found the use of modified BPM reduced turn-around time of conventional blood culture which may be critical in instituting prompt antibiotic therapy.

REFERENCES

1. Weinstein MP. Current blood culture methods and systems: Clinical concepts, technology, and interpretation of results. Clin Infect Dis 1996;23(1):40-6.
2. Mir BA, Srikanth C. Prevalence and antimicrobial susceptibility of methicillin resistant *Staphylococcus aureus* and coagulase-negative Staphylococci in a tertiary care hospital. Asian J Pharm Clin Res 2013;6(3):231-4.
3. Castaneda MR. A practical method for routine blood cultures in brucellosis. Proc Soc Exp Biol Med 1947;64(1):114.
4. Koshi G, Mukundan U, Mathew M. Advantages of MacConkey biphasic medium for blood culture. Indian J Med Res 1985;81:584-90.
5. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie & McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone; 2010.
6. de Cueto M, Ceballos E, Martinez-Martinez L, Perea EJ, Pascual A. Use of positive blood cultures for direct identification and susceptibility testing with the vitek 2 system. J Clin Microbiol 2004;42(8):3734-8.