

Evaluation of Time to Detection of Bacteria and Yeasts Isolated From Blood with BACTEC 9120 Automated Culture System

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ABSTRACT

Introduction: Conventional diagnostic methods for culture of normally sterile body fluids involve their inoculation directly into agar media or into various blood culture media like Brain Heart Infusion (BHI) broth. The yield of these methods is limited due to small volume of fluid cultured, overgrowth by contaminated microflora and phagocytosis of microorganisms by white blood cells & other contributing factors. BACTEC automated culture system for microbial isolation has many advantages over the conventional culture methods such as reduced time to detection, isolation of fastidious microorganisms, simpler transportation of specimen to laboratory and microbial growth positivity even after initiation of antimicrobial therapy.

Aims and objectives: To isolate and identify the bacterial pathogens from blood & to compare the time needed for the detection of microorganisms by BACTEC 9120 microbial detection system.

Materials and Methods: The study was carried out in the Department of Microbiology, Dr. Rajendra Prasad Government Medical College (DRPGMC) & Hospital, Kangra at Tanda over a period of six months w.e.f. October, 2014 to March, 2015. Blood suspected to be infected was included in the study. Statistical analysis was done using standard statistical techniques. BACTEC system consists of self-contained incubator, agitator and detection device with capacity of 120 bottles in BACTEC 9120 model. The sample to be tested is inoculated into the vial which is entered into the BACTEC instrument for incubation and periodic reading. Each vial contains a sensor which responds to the concentration of CO₂ produced by the metabolism of microorganisms or the consumption of oxygen needed for the growth of microorganisms. The sensor is monitored by the instrument every ten minutes for an increase in its fluorescence, which is

proportional to the increasing amount of CO₂ or the decreasing amount of O₂ present in the vial. A positive reading indicates the presumptive presence of viable microorganisms. BACTEC Blood culture bottles contain soya bean casein broth & SPS (Sodium polyanethol sulfonate).

Results and Conclusions: BACTEC automated culture system for microbial isolation has many advantages such as reduced time to detection, isolation of fastidious microorganisms, simpler transportation of specimen to laboratory and microbial growth positivity even after initiation of antimicrobial therapy. The presence of resins and lytic agents like saponin in BACTEC Peds Plus /F may decrease the inhibitory effects of antibiotics and favours the release of phagocytosed organisms. Also the dilution effect of inoculum into liquid medium in BACTEC culture bottle may decrease the inhibitory effect of inhibitors present in the fluids.

Keywords: BACTEC Automated Culture System, Blood, Microbial Isolation.

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INTRODUCTION

Blood stream infections are life threatening infections leading to due consequences if not detected and treated properly. Detection and identification of blood borne pathogens should be amongst the top most priorities of any microbiology laboratory. The blood culture methods include Conventional method of blood culture and semi-automated and automated blood culture systems. One of the

automated blood culture systems include BACTEC 9120 system in which the blood culture bottles are monitored every 10 minutes for growth. Each vial contains a sensor which responds to the concentration of CO₂ produced by the metabolism of microorganisms or the consumption of oxygen needed for the growth of microorganisms. The sensor is monitored by the

instrument every ten minutes for an increase in its fluorescence, which is proportional to the increasing amount of CO₂ or the decreasing amount of O₂ present in the vial. A positive reading indicates the presumptive presence of viable microorganisms. The position of positive bottle is indicated on the computer screen. The detection of microorganisms in a patient's sterile body fluids has diagnostic and prognostic importance. A "growth index" exceeding a predefined threshold is considered evidence of microbial growth. Indications for blood culture includes pyrexia of unknown origin and septicaemia. In BHI broth after incubation turbidity is seen and after Gram staining, subcultures are done over blood agar, MacConkey agar, chocolate agar and Sabouraud's agar. After 24 hours at 37°C aerobic as well as in presence of 5-10% CO₂ growth have to be seen. If microorganisms do not grow then sometimes prolong incubation is done or subsequent subcultures are required and these all leads to more contamination and labour and less result. Results are obtained in 5-7 days after conventional methods. BACTEC automated culture system is a self-agitator closed system having in built incubator and gives less chances of contamination at the level of laboratory during processing and requires less labour due to lack of subsequent subcultures. BACTEC automated culture system for microbial isolation has many advantages over the conventional culture methods such as reduced time to detection, isolation of fastidious microorganisms and simpler transportation of specimen to the laboratory.

The present study was undertaken with the aim to isolate and identify the bacterial pathogens from blood by processing in BACTEC 9120 system and to evaluate the time needed for the detection of microorganisms by BACTEC 9120 microbial detection system.

MATERIALS AND METHODS

This cross sectional study was conducted in the Department of Microbiology of Dr. Rajendra Prasad Government Medical College (DRPGMC) & Hospital, Kangra at Tanda for a period of 6 months.

A total of 378 blood samples of patients suffering from PUO (pyrexia of unknown origin) and septicaemia were received in the Department of Microbiology in BD BACTEC Plus Aerobic/F culture vials for paediatric patients. Samples with inadequate volume of blood (< 5ml for adults and <3ml for children) was excluded from the study. BACTEC vials were processed as per standard microbiological techniques.

Taking all aseptic precautions 5-10 ml of blood from adults and 2-3 ml from paediatric patients was collected and inoculated into the respective BACTEC culture vials for adults and children. Vials were entered into the BACTEC instrument for incubation and periodic reading which occurs every 10 minutes for positive outcome. On getting an alert for a positive vial from the BACTEC instrument Grams staining was done immediately. Further the vial was subcultured on Blood agar, Chocolate agar, Sabouraud's dextrose agar and MacConkey agar. The time to detection of all the BACTEC vials giving positive result was noted and according to it all positive samples were divided in three groups (<24 hrs, 24-48 hrs & 48-120 hrs). The isolates so obtained after subculture were identified by standard microbiological techniques. Methicillin resistant Staphylococcus aureus was detected by Cephoxitin disc of 30 µg. Extended spectrum beta lactamase production amongst Gram negative bacteria was detected by cefotaxime (30µg) and ceftazidime (30µg) disks. All negative vials were incubated for a period of five days.

RESULTS

Out of a total of 378 blood samples received 134 (35.44%) were found to be positive. Males were 220 (58.2%) and 158 (41.8%) were females. Inborn patients 321 (84.92%) were more common than 57 (15.08%) outdoor patients. According to age groups the most common age group was 0-10 years followed by 21-40 years.

Table 1: Culture; time of detection

| Culture Positive | 24 Hrs | 24-48 Hrs | 48-120 Hrs |
|------------------|--------|-----------|------------|
| No | 86 | 39 | 9 |

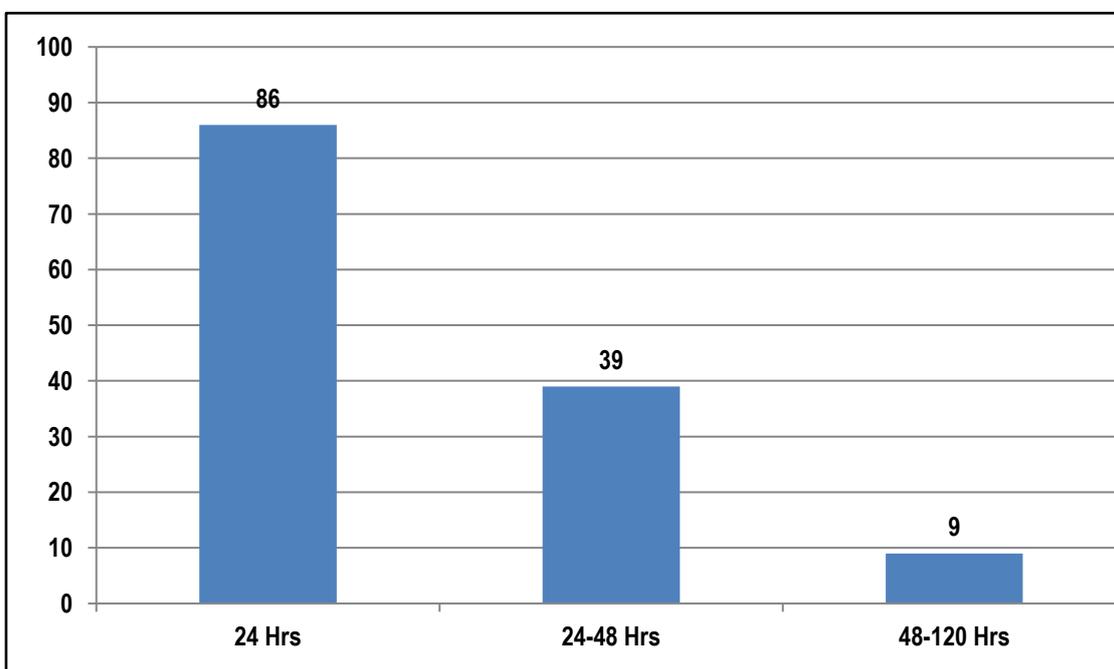


Fig 1: No. of organisms detected by BACTEC 9120 system from blood with detection time

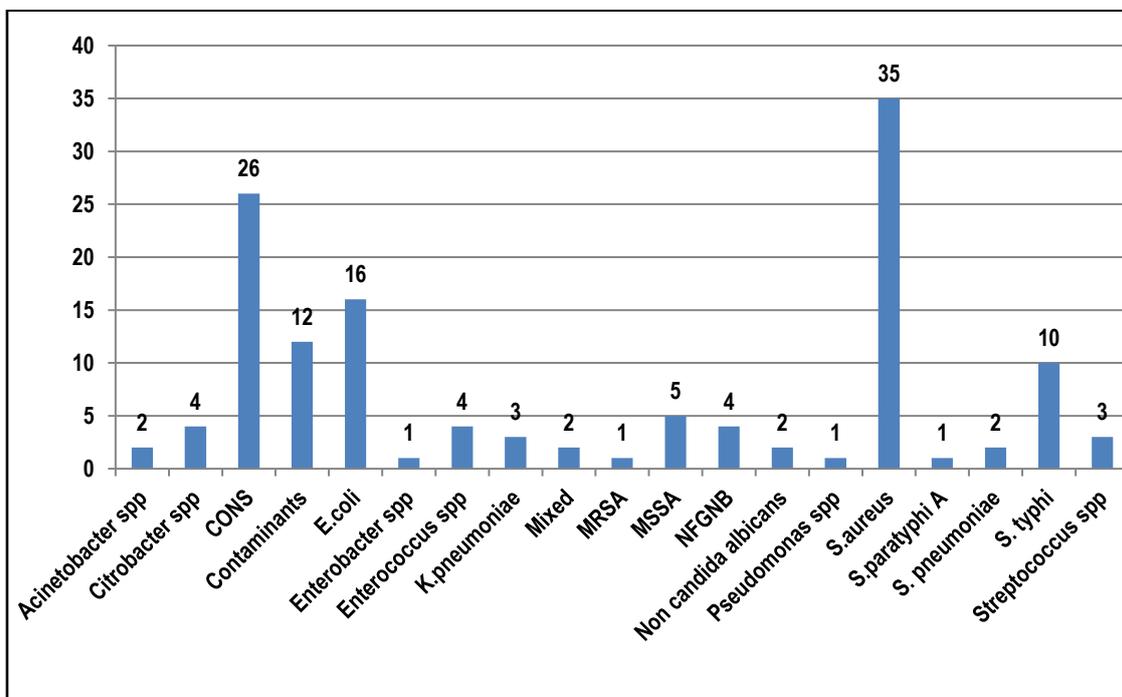


Fig 2: No. of organisms detected by BACTEC 9120 system from blood

Out of 134 positive samples, 86 (64.17%), 39 (29.10%) & 9 (6.71%) were detected in <24 hrs, 24-48 hrs & 48-120 hours respectively. The earliest time to detection (TTD) was 4 hours and latest TTD was 94 hours. (Table 1) (Fig1). Gram positive bacteria 56.71% were more commonly isolated than Gram negative bacteria 31.34%. The most common organism isolated was *Staphylococcus aureus* 35 (26.1%) in blood sample. The details of the organisms isolated are given in Table 3, Figure 2. Mixed growth was obtained in 1.49% of cases and in 8.95% of blood samples only skin commensals were obtained. *Candida* species were obtained in 1.49% cases. Superbugs methicillin resistant *Staphylococcus aureus* and extended spectrum beta lactamases producing microorganisms were identified in 29.26% and 42.85% respectively. All the gram positive bacilli were sensitive to vancomycin. Other organisms detected were *S. typhi*, *S. paratyphi A*, Non-fermenters etc.

DISCUSSION

Pyrexia of unknown origin and septicaemia remain a significant cause of morbidity and mortality in all age groups. The clinical diagnosis is not sufficient to treat patients and culturing of blood specimens remains a top priority for every microbiological laboratory. Blood culture system can be of conventional, semi-automated and automated systems. Continuously monitored blood culture systems like ESP, BacT/Alert and BACTEC have been shown to be very reliable.¹⁻³ The incubation period in all these systems can be programmed according to need, the average being 5-7 days. According to previous studies, incubation for five days is sufficient in identification of bacterial pathogens in majority of cases as was done with ESP and BacT/Alert systems.^{4,5} This study was undertaken with the aim to see the time to detection and to review the 5 day incubation of blood cultures processed in BACTEC 9120 system. In our study it was shown that 64.17% of clinically significant bacterial pathogens were recovered within 24 hours of incubation. With 48 hours of incubation 93.27% organisms gave a positive alert. This is in accordance with other

studies conducted in BACTEC 9120 system.^{6,7} Reimer et al, Baka et al reported a culture positively as 97.37%, 98.5% and 97.81% respectively after 4 days of incubation.⁶⁻⁸ This is in accordance with our study. In our study clinically significant Gram positive bacterial isolates were 56.71%, which has similar results with Nita et al.⁸ CONS was considered as a significant pathogen in our study it is increasingly being reported to cause significant BSI (blood stream infections) from all over the world. Another significant finding was noted that all *Candida* species were identified within 4 days of incubation. So based on our study we do not recommend a longer incubation period of more than 5 days for growth of fastidious organisms in blood specimens. The limitations of our study included the inability to detect the volume of blood inoculated in each bottle and relatively higher contamination rates. So we recommend a 5 day incubation period protocol for the culture of all blood specimens in processing for BACTEC 9120 system for isolation of bacteria and yeasts. We also recommend that findings of Gram staining from positive vials must be immediately conveyed telephonically to the clinicians about critically ill patients. It will help in giving an early diagnosis to clinicians and initiation of appropriate treatment. This could certainly lead to a decrease in mortality and morbidity.

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