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COMPARISON OF THE BACTEC BLOOD CULTURE SYSTEM VERSUS CONVENTIONAL METHODS FOR DIAGNOSIS OF SEPTICEMIA IN NEWBORN AT TERTIARY CARE HOSPITAL JAIPUR, RAJASTHAN.

Microbiology

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ABSTRACT

Background: Seventy-six newborn admitted at NICU were selected to compare the BACTEC 9050 blood culture system and the conventional method.
Methods: This comparative study was conducted at the department of microbiology, NIMS, Jaipur from November 2018 to October 2019, and comprised blood samples of suspected newborns. The blood samples were inoculated into automated BACTEC 9050 culture bottles. At the same time, a conventional blood culture bottle was also inoculated for comparison. The antibiotics used for susceptibility testing were penicillin (P), Linezolid, Vancomycin, Ampicillin, Azithromycin, Ceftazidime, Amikacin, Gentamycin, Tetracycline, Clindamycin, Ciprofloxacin, Teicoplanin, Cotrimoxazole, Meropenem, Imipenem, Piperacillin-Tazobactam, Levofloxacin, Cefoxitin, High-Level Gentamycin, Tobramycin.
Result: Out of 76 blood culture samples, 52 (68.42%) were positive on BACTEC 9050, while 47 (61.84%) were positive through the conventional method. Out of 47 positive samples, the isolated organisms were *Escherichia coli* (09), Skin commensal (10) followed by *Staphylococcus aureus* (11), *Klebsiella pneumonia* (07), *Streptococcus* (02), *Enterococcus* (04), *Acinetobacter* (2) and *Pseudomonas aeruginosa* (02).
Conclusion: Diagnosis of newborn septicemia through BACTEC 9050 was quicker with high sensitivity compared to the conventional method.

KEYWORDS

BACTEC 9050, NICU, Newborn.

INTRODUCTION:

Sepsis is a potentially deadly hospital condition characterized by the whole body's inflammatory state which is due to a variety of pathogenic organisms in the blood (bacteria, fungi, viruses, protozoa)[1]. Sepsis is one of the very important causes of infants and child mortality. Gram-negative sepsis is usually associated with relatively higher mortality than gram-positive one if left untreated [2-3]. Blood culture is the gold standard for identifying the causative factors of blood infection. Identification of bacteria and fungi by blood culture in patients with sepsis is essential for proper treatment and selection of appropriate antibiotics [4]. Knowledge-based science in microbiology, adequate experience, employing the latest resources of microbiology, utilization of the now a day's World Health Organization protocols and standard reference laboratories are very important for accurate identification of bacteria[5]. The alarming increase in sepsis-associated mortality and drawbacks of conventional methods require a novel bacteriologic diagnostic tool with higher produce and speed in terms of accuracy and precision of reliability of results[6]. In spite of recent advancements in diagnostic molecular techniques for microbiological diagnosis of sepsis, conventional blood culture is still the gold standard. However, it has some limitations, i.e. prolonged time of reporting and a high rate of contamination. Fully automatic BACTEC methods are superior to conventional methods in terms of speed and sensitivity[7]. The conventional method includes two week culture in order to enable the slow growth of microorganism are cultured on specific media[8]. The BACTEC culture method is the simplest way of blood cultures where the fluid bottles of blood cultures with a comparable vacuum are utilized. The blood is transferred to the blood culture bottle in sterile conditions, it is turned pull down for a few minutes, a hole is created in its cover using a sterile needle and it is placed in an incubator. This medium is commonly used for the identification of bacteria[9]. If the glasses of automated blood culture system of BACTEC inform microbiologists when the growth level is enough to reach the level that is detectable by the device, then it is important for quick decision making for patients[10]. There is limited available data from developing countries to evaluate the performance of automated blood cultures through BACTEC 9050 in comparison with conventional ones for the diagnosis of pediatric septicemia. In the present study was prepared to determine the utility of automated blood culture system BACTEC 9050 for the detection of clinically significant pathogens in blood and its comparison with conventional blood culture.

INCLUSIVE CRITERIA

Pediatric patients aged >1 months, who had symptoms of septicemia were included in this study.

EXCLUSION CRITERIA

Antibiotic therapy patients are excluded in this study

MATERIAL AND METHODS:

A prospective, cross-sectional study was conducted in the microbiology department. Among 76 pediatric patients who were admitted at NICU of NIMS medical college hospital, Jaipur, Rajasthan, India.

Blood collection was done under aseptic condition; disinfect the vein puncture site using chlorhexidine with 70% alcohol swabs, allowing the site to get dry completely. 0.5 to 1 ml of blood was drawn and placed on a pediatric aerobic bottle collected from NICU.

BD BACTEC 9050 system was used for incubation and the bottle was incubated until microbial growth was detected. BACTEC 9050 is an automated blood culture system, which contains a sensor which response to the concentration of CO_2 produced by the metabolism of microorganism or consumption of O_2 needed for the growth of microorganism. The sensor is monitored by the instruments every ten minutes for an increase in its fluorescence which is proportional to the increasing amount of CO_2 or decreasing the amount of O_2 present in the vial. BACTEC 9050 bottles that showed the growth were plated into sheep Blood agar and MacConkey Agar and further incubated at 35+/- 2ºC. Growths were stained by Gram staining method[11]. The positive growth was farther processed by routine biochemical reactions and antibiotic susceptibility was put up by modified Kirby Bauer's method[12]. Following antibiotics were used for antibiotic susceptibility testing followed by Antibiotic used in present study were Penicillin (P), Linezolid (LZ), Vancomycin (VA), Ampicillin (AMP), Azithromycin (AZM), Ceftazidime (CAZ), Amikacin (AK), Gentamycin (GEN), Tetracycline (TE), Clindamycin (CD), Ciprofloxacin (CIP), Teicoplanin (TEI), Cotrimoxazole (COT), Meropenem (MRP), Imipenem (IPM), Piperacillin-Tazobactam (PIT), Levofloxacin (LE), Cefotaxime(CX), High Level Gentamycin(HLG), and Tobramycin (TOB).

RESULTS:

Table 1: Blood culture showed 47(61.84%) positivity and 29(38.16%)

negativity by conventional method and 52 (68.42%) positivity and 24(31.58%) negativity by BACTEC 9050. The average time of detection of microbial growth was 24 hours in the conventional method and 18 hours in the BACTEC system for the same.

Table 1: Comparison of Culture positivity and negativity and time of detection of conventional and BACTEC method of Blood culture (n=76)

Blood culture method	Culture Positivity	Culture negativity	Time of detection
Conventional	47(61.84%)	29(38.16%)	24 hours
BACTEC	52 (68.42%)	24(31.58%)	18 hours

Table 2: The study of the organism isolated from the conventional culture methods was *Staphylococcus aureus* (23.40%), *E. coli* (19.15%), *Klebsiella spp.* (14.89%), *Enterococcus spp.* (8.51%), *Pseudomonas aeruginosa* (4.26%), *Acinetobacter spp.* (4.26%), *Streptococcus spp.* (4.26%), and Skin commensal (21.17%).

Table 2- Etiological agent associated with sepsis		
Name of Organism	**Isolation with**	**Percentage (%)**
Staphylococcus aureus	11	23.40 %
E.coli	09	19.15%
Klebsiella spp	07	14.89%
Enterococcus	04	8.51%
Pseudomonas aeruginosa	02	4.26%
Acinetobacter spp	02	4.26%
Streptococcus spp.	02	4.26%
Skin Commensal	10	21.27%
Total	**47**	**100%**

Table 3 showed *staphylococcus aureus* Linezolid was the most sensitive drug followed by Vancomycin, Cefoxitin, Levofloxacin, Azithromycin, and Ciprofloxacin while most resistant drugs were Penicillin, Gentamycin, and Clindamycin. For *Enterococcus spp*. Linezolid was most sensitive drug followed by High-level Gentamycin, Levofloxacin, Tetracycline and Teicoplanin while most resistant drugs were Vancomycin and Penicillin. For *Streptococcus spp*. Linezolid was the most sensitive drug followed by Vancomycin, Azithromycin, Levofloxacin, and Tetracycline while the most resistant drug was penicillin.

Table 3: Antibiotic susceptibility pattern of Gram Positive organisms

Staphylococcus (n=11)			Enterococcus (n=4)			Streptococcus (n=2)		
Antibiotics	**Sensitive**	**Resistance**	**Antibiotics**	**Sensitive**	**Resistance**	**Antibiotics**	**Sensitive**	**Resistance**
Cefoxitin	08	03	High Level Gentamycin	03	01	Azithromycin	02	00
Linezolid	10	01	-	-	-	Linezolid	02	00
Vancomycin	09	02	Vancomycin	01	03	Vancomycin	02	00
Levofloxacin	08	03	Linezolid	04	00	Levofloxacin	02	00
Ciprofloxacin	07	04	Levofloxacin	03	01	Ciprofloxacin	01	01
Azithromycin	08	03	Ciprofloxacin	02	02	Amikacin	00	02
Gentamycin	05	06	Penicillin	01	03	Gentamycin	01	01
Clindamycin	06	05	Ampicillin	02	02	Clindamycin	01	01
Penicillin	04	07	Tetracycline	03	01	Penicillin	00	02
Tetracycline	09	02	Teicoplanin	03	01	Tetracycline	01	01

Table 4 Showed among all Gram-Negative organisms Colistin was the most sensitive drug followed by Meropenem and Levofloxacin while most resistant drugs were Gentamycin and Ciprofloxacin.

Table 4: Antibiotic susceptibility pattern of Gram Negative organisms

Escherichia coli (n=09)			*Klebsiella Spp. (n=07)*			*Pseudomonas Aeruginosa (n=02)*			*Acinetobacter spp. (n=02)*		
Antibiotics	**Sensitivity**	**Resistance**	**Antibiotics**	**Sensitivity**	**Resistance**	**Antibiotics**	**Sensitivity**	**Resistance**	**Antibiotics**	**Sensitivity**	**Resistance**
Ceftazidime	03	06	Ceftazidime	04	03	Ceftazidime	01	01	Ceftazidime	0	2
Gentamycin	02	07	Gentamycin	02	05	Gentamycin	00	02	Gentamycin	0	2
Tobramycin	-	-	Tobramycin	05	02	Tobramycin	01	01	Tobramycin	1	1
Piperacillin-Tazobactam	05	04	Piperacillin-Tazobactam	04	03	Piperacillin-Tazobactam	01	01	Piperacillin-Tazobactam	0	1
Amikacin	06	03	Amikacin	05	02	Amikacin	01	01	Amikacin	0	1
C o - trimoxazole	05	04	C o - trimoxazole	03	04	Aztreonam	02	00	C o - trimoxazole	0	2
Levofloxacin	07	02	Levofloxacin	06	01	Levofloxacin	01	00	Levofloxacin	1	1
Ciprofloxacin	03	06	Ciprofloxacin	02	05	Ciprofloxacin	01	01	Ciprofloxacin	0	2
Meropenem	07	02	Meropenem	06	01	Meropenem	02	00	Meropenem	2	0
Imipenem	06	03	Imipenem	04	03	Imipenem	01	01	Imipenem	1	1
Colistin	09	00	Colistin	06	01	Colistin	02	00	Colistin	2	0

DISCUSSION:

In this study, 52 (68.42%) samples out of 76 total samples showed growth of micro-organisms by the BACTEC system. On the rather, 47 (61.84%) samples out of 76 total samples showed growth by the conventional method. This result is similar to the studies done by other researches[13-15]. The culture-positive 23 samples by conventional blood culture positive were also showed positivity by an automated method. No isolate detected only by the conventional method and not by the BACTEC system. A study done by Emel e*t al* supports our findings[16]. In our study, the average time of growth of microorganisms is 24 hours in the conventional system and 18 hours in the automated system. The time taken to reveal a positive blood culture is very important. Adrienne *et al* [17] also reported that early recognition of bacteremia followed by prompt initial management was essential for the prevention of progression of the condition of the patient to the more severe form. Early diagnosis also helped in preventing sepsis-related disability and death. In the present study, isolated micro-organisms were *Staphylococcus aureus* (23.40%), *E. coli* (19.15%), *Klebsiella spp.* (14.89%), *Enterococcus spp*. (8.51%), *Pseudomonas aeruginosa* (4.26%), *Acinetobacter spp*. (4.26%), and Skin commensal (21.17%). These findings were more or less in agreement with previous studies [18-19]. While using this automated system, care should be taken to collect optimum volume of blood samples (1–3 ml), because optimum volume of blood can neutralize inhibitory effect of Sodium Polyanethol Sulfonate (SPS) present in BD BACTEC PED PLUS/F culture vials and allow growth of SPS sensitive and fastidious organisms from blood samples. The adequate blood sample is also required to provide growth factors, such as Nicotinamide adenine dinucleotide to certain Haemophilus species. Lin *et al*[20], reported that optimum blood volume obtained from the patient was directly proportional to the growth of microorganisms in the automated blood culture system. Some other researchers observed that the use of less than 1 ml of blood in neonates often shows a false negative result. They found several plus points for automated blood culture systems, such as the higher recovery of etiological microorganisms, fully automated, and easy method of operation. But at the same time, they also reported high implementation cost and requirement of continuous power supply [21]. The sample size was relatively small due to limited time and scarce

resources. Moreover, the data presented was the result of our initial program attempts to evaluate the diagnostic accuracy of BACTECT 9050 in pediatric sepsis. We recommend more such studies to prove the diagnostic efficacy of the BACTECT automated culture system in the diagnosis of sepsis.

CONCLUSION:

Both BACTEC and conventional methods have high validity. To evaluate the results of blood culture and infection control, experts can use either of these methods to study the results of bacterial blood culture. Due to the high cost of automated systems compared to manual methods, blood culture is performed in most health care centres. These methods have the advantage of being cheap, readily available, and not requiring sophisticated and expensive devices.

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