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Comparative Evaluation of Conventional Blood Culture with Bactec 9050 for Bacterial Isolates in Clinically Suspected Cases of Fever of Unknown Origin

Avneet Kaur[1], Puneet Singh Soodan[2], Varsha A Singh[3]
[1](*Microbiology, M.M.Institute of Medical Sciences and Research , Mullana, Ambala/ Maharishi Markandeshwar University, India)*
[2](*Dermatology, Shri Ram Murti Smarak institute of Medical Sciences, Barielly (U.P)/Rohilkhand University,India)*
[3](*Microbiology, M.M.Institute of Medical Sciences and Research , Mullana, Ambala/ Maharishi Markandeshwar University, India)*

Abstract:
Background*: Patients with fever of unknown origin (FUO) are elusive and challenging clinical cases. Timely detection and identification of blood borne pathogen is one of the most important functions of microbiology laboratory.*
Objectives: *To evaluate the efficacy of BACTEC 9050 in comparison to conventional blood culture for detection of bacterial isolates in clinically suspected cases of fever of unknown origin.*
Material and methods: *Blood samples from 100 suspected cases of fever of unknown origin (FUO) were subjected to conventional blood culture and BACTEC 9050 culture system..*
Results: *Out of 100 suspected cases, 46% cases were culture positive with 80.43% pathogenic bacterial isolates comprising of 54.05% gram positive and 45.94% gram negative isolates. Predominant gram positive isolates were 35% coagulase negative Staphylococcus and 30% Staphylococcus aureus. Gram negative isolates were 29.41% Salmonella typhi followed by 17.64% E coli. BACTEC 9050 was observed to be sensitive (100%) as compared to conventional blood culture (67.56%) . Mean time to detection of significant pathogens was significantly less with the BACTEC 9050 than with conventional media.*
Conclusion: *BACTEC 9050 proved as a reliable and fast method to identify the blood stream pathogens in blood culture as compared to conventional culture methods.*
Keywords: *BACTEC 9050- Becton Dickinson Microbiology Systems, FUO- fever of unknown origin*

I. Introduction

Pyrexia of unknown origin (PUO) continues to be one of the most challenging situations facing the physician.[1] It is identified as a syndrome of fever that does not resolve spontaneously and in which the cause remains elusive even after an extensive diagnostic workup. Petersdorf and Beeson first coined the term fever of unknown origin in 1961 and explicitly defined it as (1) Temperature >38.3ºC (101ºF) on several occasions (2) duration of fever of more than 3 weeks and (3) failure to reach to diagnosis despite one week of inpatient investigations.[2]The differential diagnosis for PUO comprises over 200 disorders and is among the longest of any condition in medicine.[3] In adults, infections and cancer account for about 25-40% cases each of PUO.[4] Autoimmune disorders account for rest of the 10-20%cases.[5] In children, 30-50% cases are due to infections, 5-10% cancer and remaining 10-20% cases are due to autoimmune disorders. A cause is never identified in a significant proportion (19%) of patients.[6] Infectious disease is one of the leading causes of morbidity and mortality in most developing countries. A meticulous history, a thorough physical examination, discriminative use of investigative procedures and constant reassessment of all the parameters reveal the cause of the patients fever.[7] Despite recent developed techniques, like nucleic acid probes, PCR and other molecular techniques for microbiological diagnosis, blood culture still remains the most practical and reliable method in the diagnosis of bloodstream infections.[8] Blood cultures provide the best yield for microbiological diagnosis, with sensitivity ranging from 53 to 90%.[9] Instrumentation of blood culture has accomplished rapidness, accuracy and cost effectiveness. It also eliminates cross contamination of cultures during repeated subculture. The BACTEC 9000 series of blood culture systems are fluorogenic, automated, non-invasive blood culture system designed for processing three to five blood cultures per day. [10] Conventional method includes culture for two weeks to detect slow growing organisms and on special media if necessary. Hence our study was an attempt to evaluate the capability, efficiency and reliability of BACTEC 9050 in comparison to conventional blood culture for detection of bacterial isolates in clinically suspected cases of fever of unknown origin.

II. Material & Methods:

This cross-sectional study was carried out during the period January 2011 to December 2011 at M.M.Institute of Medical Sciences and Research , Mullana. Blood samples from 100 suspected cases of fever of unknown origin (FUO) attending the OPD and indoor were included in the study. Diagnosed cases of fever were excluded.

2.1 Sample Collection And Processing:

10 ml blood was collected aseptically from adult patients and was divided equally into BACTEC blood culture vial (aerobic) and conventional blood culture bottle containing 50ml of brain heart infusion broth (Dilution 1:10).[11] For paediatric patients, 2 ml of blood was collected and equally transferred into the BACTEC™ PEDS PLUS/F vial and Conventional blood culture bottle containing 10ml of brain heart infusion broth.[12]The inoculated BACTEC vials and conventional blood culture bottles were transported to the laboratory immediately.

The BACTEC bottle was placed into the BACTEC 9050. On receiving positive signals, bottles were removed and an aliquot of the broth was gram stained and subcultured onto the Blood agar, Mac Conkey's agar and Chocolate agar. Organism were identified by battery of biochemical reaction and all bottles were incubated for a minimum of 7 days before labelling as negative as per the manufacturer's protocol.[10]All negative bottles were subcultured to chocolate agar plates and incubated aerobically at the end of the incubation period.Conventional technique was done by inoculating blood into blood culture bottles containing brain heart infusion broth. The bottles were incubated at 37 ^{0} C and were shaken periodically. On 3rd, 5th, and 7th day, subcultures were done on Blood agar, Mac Conkey's agar and Chocolate agar. Futher processing was done by standard laboratory procedures.[13] The negative blood sample was discarded after 7 days of incubation.

III. Result

The present study was a cross sectional study carried out at Bacteriology division in the Department of Microbiology at M.M.Institute of Medical Sciences & Research, Mullana over a period of one year from January 2011 to December 2011. In this study an attempt has been made to compare BACTEC 9050 with Conventional blood culture for detecting pathogenic bacterial isolates.

Table 1: Rate of culture positivity in suspected patients of Fever of unknown origin

Total (n)	Culture positive n(%)	Sterile n(%)
100	46 (46)	54 (54)

Out of total of 100 samples of fever of unknown origin culture positivity was seen in 46 (46%) cases and 54 were sterile cases (54%) by either of the two methods i.e. conventional blood culture and BACTEC 9050.

Table 2: Distribution of Blood culture isolates on the basis of pathogenicity

Total (n)	Pathogenic isolates n (%)	Non pathogenic isolates n (%)
46	37 (80.43)	9 (19.56)

Out of 46 culture positive isolates, 37 (80.43%) isolates were pathogenic while remaining 9 (19.56%) were non pathogenic isolates.

Table 3 : Distribution of pathogenic organisms in Blood culture

Total (n)	Gram positive n (%)	Gram negative n(%)
37	20(54.05)	17(45.94)

Among the pathogenic isolates, 20 isolates (54.05%) were gram positive while remaining 17 isolates (45.94%) were found to be gram negative.

Table 4: Distribution of Pathogenic Gram positive organism

Total (n)	coagulase negative Staphylococcus n (%)	Staphylococcus aureus n (%)	Streptococcus pneumonia n (%)	Enterococcus n (%)	Candida n (%)
20	7(35)	6(30)	3(15)	2(10)	2(10)

Out of 20 gram positive cases, 7 (35%) were coagulase negative Staphylococcus followed by 6 (30%) Staphylococcus aureus, 3(15%) were Streptococcus pneumonia, Enterococcus 2(10%) and Candida 2(10%).

Table 5: Distribution of Pathogenic Gram negative organism

Total (n)	S.typhi n(%)	Ecoli n(%)	Pseudomonas n (%)	Klebsiella n (%)	Acinetobacter n (%)	Citrobacter n (%)	Brucella n (%)
17	5 (29.41)	3 (17.64)	3 (17.64)	3 (17.64)	1 (5.88)	1 (5.88)	1 (5.88)

Out of 17 cases, 5 (29.41%) were Salmonella typhi followed by E coli 3(17.64%), Pseudomonas 3(17.64%), Klebsiella 3(17.64%), Acinetobacter 1(5.88%), Citrobacter 1(5.88%) and Brucella 1(5.88%).

Table 6: Distribution of culture positive cases according to Gender, Age and Residence

Parameters	Culture positive (n=46)	(%)
Gender		
Male	30*	65.22
Female	16	34.78
Age		
<20	24*	52.17
21-30	6	13.04
31-40	10	21.73
41-50	4	8.69
>50	2	4.34
Social status		
Urban	16	34.78
Rural	30*	65.22

*indicates 1 positive sample of Brucella.

In present study, males constituted majority (65.22%) of the patients from rural background (65.22%). No definite correlation between occupation and culture positivity was observed .Maximum patients were found in the younger age group of less than 20 years (52.17%) followed by 31-40 years (21.73%) , 21- 30 years (13.04%), 31- 40 years (8.69%) and > 50 years of age (4.34%).

Table 7: Total time of detection of Bacterial isolates by BACTEC 9050

Type of organism	No of samples n(%)	Detection times (Hours)		
		Max	Min	Mean
All Gram Positive Bacteria	**20**	**-**	**-**	**19.33**
Coagulase negative staphylococcus	7(35)	**38**	**7.3**	**32.51**
Staphylococcus aureus	6(30)	**43**	**2.2**	**13.23**
Streptococcus pneumonia	3(15)	**12.25**	**3.2**	**10.05**
Enterococcus	2(10)	**36**	**12.3**	**21.32**
Candida	2(10)	**29.53**	**22**	**24.04**
All Gram Negative Bacteria	**17**	**-**	**-**	**19.06**
Pseudomonas	3 (17.64)	**46**	**8.3**	**22.07**
Klebsiella	3 (17.64)	**15**	**12**	**13.3**
Acinetobacter	1 (5.88%)	**19.3**	**12**	**18.47**
Citrobacter	1 (5.88)	**13.05**	**8.5**	**10.77**
Brucella	1 (5.88)	**96**	**168**	**132**

The mean detection time taken by BACTEC 9050 for gram-positive bacteria and gram-negative bacteria in this study were 19.33 hours and 19.06 hours respectively whereas time taken for detection of Brucella isolates was minimum of 4 days (96hrs) and maximum of 7 (168)days

Table 8: Total time of detection of Bacterial isolates by Conventional blood culture methods

Method	Time taken for positivity	
	Gram positive bacteria (n=20)	Gram negative bacteria(n=17)
Conventional blood culture	5-7 Days	5-7 Days

Total time taken for detection of bacterial isolates by conventional methods was upto 5-7 days with repeated subcultures.

Table 9: Distribution of Pathogenic isolates by Conventional blood culture and BACTEC 9050.

Pathogenic isolates (n)	Conventional blood culture (n)	BACTEC 9050 (n)
Coagulase negative Staphylococcus (7)	6	7
Staphylococcus aureus (6)	4	6
Streptococcus pneumonia (3)	2	3
Enterococcus (2)	1	2
Candida (2)	2	2

Salmonella typhi (4)	2	4
E.coli (4)	2	4
Pseudomonas (3)	2	3
Klebsiella(3)	2	3
Acinetobacter (1)	1	1
Citrobacter (1)	1	1
Brucella (1)	-	1

Maximum pathogenic isolates detected by both conventional blood culture and Bactec 9050 were coagulase negative Staphylococcus and Staphylococcus aureus. The highest rate of recovery of pathogenic isolates was by BACTEC 9050 i.e. 100% (37/37) as compared to conventional blood culture methods 67.56% (25/37). There were 12 samples (32%) which were found to be positive only by BACTEC 9050 and not by Conventional blood culture methods. One positive sample of Brucella (3%) was detected by BACTEC 9050 only.

Table 10: Efficacy of BACTEC 9050 with respect to Conventional blood culture methods

Blood culture	**BACTEC**		**Total**
	+	**-**	
+	**25(TP)**	**0(FN)**	**25**
-	**12(FP)**	**63(TN)**	**75**
	37	**63**	**100**

TP= TRUE POSITIVE
FN= FALSE NEGATIVE
FP= FALSE POSITIVE
TN= TRUE NEGATIVE
SENSITIVITY= [TP / (TP+FN)]* 100
SPECIFICITY= [TN /(TN+FP)]*100
POSITIVE PREDICTIVE VALUE= [TP/ (TP+FP)]*100
NEGATIVE PREDICTIVE VALUE= [TN/ (TN+FN)]*100
SENSITIVITY= [25/ (25+0)]* 100= 100%
SPECIFICITY= [63/ (63+12)]* 100= 84%
POSITIVE PREDICTIVE VALUE= [25/ (25+12)]* 100= 67.56
NEGATIVE PREDICTIVE VALUE= [63/ (63+0)]*100= 100%

The Sensitivity,Specificity,Positive Predictive value and Negative predictive value of BACTEC 9050 was found to be 100%,84%,67.56% and 100% respectively against conventional culture.

IV. Discussion

Patients with fever of unknown origin (FUO) are elusive and challenging clinical cases. Timely detection and identification of blood borne pathogen is one of the most important functions of microbiology laboratory. In the present study, blood culture positivity was seen in 46% cases with 37% pathogenic isolates comprising of 54.05% gram positive and 45.94% gram negative bacteria. These results are consistent with the study done by Jung *et al* (1999)[7] and Handa *et al* who reported 43.8% infectious causes of FUO.[14] In present study, maximum isolates of gram positive bacteria were coagulase negative Staphylococcus (35%) followed by Staphylococcus aureus (30%), Streptococcus pneumonia (15%), Enterococcus (10%) and Candida (10%). While gram negative bacteria comprised mainly of Salmonella typhi (29.41%) followed by E coli (17.64%), Pseudomonas (17.64%), Klebsiella (17.64%), Acinetobacter (5.88%), Citrobacter (5.88%) and Brucella (5.88%). These findings are in agreement with study done by Gopi *et al* (2011) who reported that among (61.52%) gram positive bacteria, maximum were coagulase negative Staphylococcus (29.92%) while gram negative bacteria (36.94%) comprised mainly of Enterobacteriacea (25.34%) i.e. Salmonella typhi were (14.35%), E coli (2.90%), Klebsiella (1.06%), Citrobacter (1.37%) and Acinetobacter (5.80%).[15] However contrary to present study, Durmaz et al (2003) reported more gram negative isolates from such FUO cases.[8] In present study, males constituted majority (65.22%) of the patients from rural background (65.22%). No definite correlation between occupation and culture positivity was seen. This finding was similar with Asmaa *et al* (2005) who reported (66.66%) male and (33.33%) female and found male to female ratio of 1.99:1[16]. The increase member of male patients over female in this study might be due to occupational exposure to animals. Male are the active and main earning member of the most of the family still now, so they are more privileged to visit physician chamber for treatment.[17] Maximum patients were found in the younger age group of less than 20 years (52.17%) followed by 31-40 years (21.73%), 21- 30 years (13.04%), 31- 40 years(8.69%) and (4. 34%)

were > 50 years of age. A study by Asmaa *et al* (2005) reported most of the clinically suspected cases (43.30%) in the age group between 1-15 years having very close correlation with present study[16]. This study evaluates the capability, efficiency and reliability of BACTEC 9050 in comparison to conventional blood culture for detection of bacterial isolates. The highest rate of recovery of pathogenic isolates was by BACTEC 9050 i.e. 37 (100%) as compared to conventional blood culture methods 25 out of 37 (67.56%). There were 12 samples (32%) which were found to be positive only by BACTEC 9050 and not by conventional blood culture methods. Positive sample of Brucella 1(3%) was detected by BACTEC 9050 only. The mean detection time taken by BACTEC 9050 for gram-positive bacteria and gram-negative bacteria in this study was 19.33 hours and 19.06 hours respectively whereas time taken for detection of Brucella isolates was minimum of 4 days and maximum of 7 days. Conventional methods took upto 5-7 days to detect positive bacterial isolates with repeated subcultures.These results are consistent with the study done by Gopi *et al* (2011)[15] who reported that the mean detection time for the clinical significant isolates by BACTEC 9050 was 21 h with 9% pathogenic positive cultures comprising of 61% gram- positive and 36% gram-negative bacteria. Hence BACTEC 9050 was found to be more sensitive in detecting pathogenic bacterial isolates as compared to conventional blood culture methods.

V. Conclusion

In the end it is concluded that out of 100 samples, 46% were culture positive and 54% were sterile by either of the two methods i.e. conventional blood culture and BACTEC 9050. Highest rate of pathogenic isolates were recovered by BACTEC 9050 (100%) as compared to conventional blood culture (67.56%) indicating sensitivity and accuracy of BACTEC 9050 for culturing the microorganism in clinical specimens. Futhermore, mean time to detection of significant pathogens was significantly less with the BACTEC 9050 than with conventional media. 80.43% pathogenic isolates were detected with (54.05%) gram positive and (45.94%) gram negative isolates. Maximum gram positive isolates were coagulase negative Staphylococcus (35%) and Staphylococcus aureus (30%) while gram negative bacteria comprised mainly of Salmonella typhi (29.41%) and E coli (17.64%) . Hence BACTEC 9050 has high sensitivity, specificity, PPV and NPV as compared to conventional blood culture examination..

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