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"COMPARATIVE EVALUATION OF CONVENTIONAL AND AUTOMATED CULTURE SYSTEM FOR DETECTION OF SEPTICEMIA IN A TERTIARY CARE HOSPITAL: A PROSPECTIVE STUDY"

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ABSTRACT **Background:** - Blood culture is the gold standard for identifying the blood stream infections (BSI) such as endocarditis, pneumonia and pyrexia of unknown origin. Automated blood system can be compared with conventional blood system in terms of quality. Accurate diagnosis of bacteria and appropriate antibiogram of BSI enhances early efficient treatment and promote public health. **Aim & Objectives: -** This study is aimed to compare blood samples of suspected bacteremia which are processed with both conventional culture methods and BACTEC FX40 (Automated blood culture system). **Material & Method:** - Between December 2021 to May 2022, A total 100 blood culture samples were collected and processed by the conventional and automated system for comparison. Samples suspicious of growth as indicated by the instrument were sub cultured on Blood, Chocolate and MacConkey agar and incubated at 37oC. Identification done by conventional and VITEK-2 system. **Results:** - Out of 100 blood culture samples processed, Overall blood culture positivity was found to be 46% by automated and 24% from conventional blood culture. Out of total positive sample by automated system (46) gram negative isolates 36(78.26%) found to be predominant than gram positive 10(21.74%) found; by conventional system (24) gram negative isolates 20(83.33%) found predominant than gram positive 4(16.66%) found. Acinetobacter baumannii 11(23.91%) and Klebsiella pneumoniae 11(23.91%) were predominant organisms isolated by automated system followed by E. coli 7(15.22%) and others. **Conclusion:** - Automated blood culture system average time of all isolates were 12.18 h than of 72.26 h conventional system so in our study found that BACTEC is way faster than conventional system. Automated systems can save great time, improve quality and reduce many humanly errors in compare to conventional system which can be use in early management of critically ill patients and hasten the antibiotic therapy.

KEYWORDS : Blood culture, BSI, Automated system, Conventional System, gram-negative organisms.

INTRODUCTION

Blood stream infections are an important cause of morbidity and mortality of patients, mainly in developing countries.[1]

In the world, about 30 million people affect from blood stream infection with 3 million newborns and 1.2 million children suffering from sepsis annually.[2]

Both Gram positive and Gram-negative bacteria can cause blood stream infections. Predominance of either the Gram-positive or Gram-negative bacterial isolates is influenced by geographical location. Major Gram-negative isolates causing septicemia are *Acinetobacter* spp, *Klebsiella* spp. *Escherichia coli, Pseudomonas spp, Enterobacter* spp., *Burkholderia* spp. and Gram-positive bacteria are *Staphylococcus aureus*, *Coagulase Negative Staphylococcus, Streptococcus spp. and most common fungus causing blood stream infections are Candida spp.*[3,4]

Among the all types of nosocomial infections, bloodstream infections are a very dangerous health problem in hospital wards globally.[5]

If not treated, bloodstream infections may involve other organs leading to their damage and ultimately death.[6]

So reliable and accurate diagnosis of these infections is very important. Despite recent advances such as polymerase chain reaction (PCR) and other molecular techniques for microbiologic diagnosis, blood culture remains the most practical and most reliable method for diagnosis of blood infections with a sensitivity of 35–90%. It is fast, affordable, and precise method.[7]

An automated blood culture system is a new hope in the diagnosis of bloodstream infection as it monitors continuously with higher sensitivity, specificity and faster turnaround time.[8]

Due to unavailability of automated blood culture system at some institutes (due to high cost as compared to the conventional method), the results of the present research can be used to choose the best method suitable for blood culture. Fortunately, from 2022 onwards in a government scheme (CHIRANJIVI YOJNA) by government of Rajasthan, both conventional and automated blood culture are free in our institute. Here in this study, we compared the conventional blood culture system with the automated blood culture system BACTEC FX40 with reference to bacteriological outline and differential time to positivity. The BACTEC FX40 Automated Blood Culture System is an automated blood culture instrument used for the growth and detection of organisms present in blood samples. It uses BD's unique resin media, which neutralizes antimicrobials present in the blood allowing for faster recovery of organisms.

MATERIALS & METHODS

A prospective study of 6 months duration from December 2021 to May 2022 was conducted at MBS Hospital, Kota, Rajasthan. With consent of patient a total of 100 blood samples were collected and processed by both conventional and automated system for comparison. Approval for this study was obtained by order no. F.3() Acad/Ethical Clearance/2021/44 Dated 15.03.2021 from the institutional ethics committee.

Inclusion Criteria: - All the patients with signs and symptoms of septicemia like fever, malaise, chills, tachycardia and hyperventilation were included.

Exclusion Criteria:

- Patient on antimicrobial therapy
- Tuberculosis patient
- Any yeast.
- Blood cultures showing mixed growth (>1 types)

Specimen Collection: Firstly, suitable vein from which blood was drawn was disinfected by two-way method 70% ethanol and then 2% tincture iodine was applied in circular motion and allowed it for air drying for 1 minute. If a patient had an existing IV line, the blood was drawn below the existing line; blood drawn above the line were to diluted with fluid being infused.

Specimen Volume: In adults 20 ml of blood (10 ml for automated blood culture bottle and 10 ml for conventional blood culture bottle)

and in children 2-4 ml of blood (2 ml for automated blood culture bottle and 2 ml for conventional blood culture bottle) was collected. After inoculating the blood into bottle, it was gently shaken to mix.

Laboratory Procedure: Bottles were transported to the laboratory for further processing. Bottles were inspected visually for damage, leakage or deterioration. The conventional and BACTEC bottles were processed according to protocol.

The conventional blood culture bottles were incubated aerobically at 37°C. Subcultures were made at the end of 24 h, 72 h, and 7th day of incubation on 5% sheep blood agar. If no growth was detected at the end of 7 days of incubation, it was reported negative. [9,10]

In automated blood culture, bottles were loaded into the BACTEC FX40 machine. As soon as an audible or visible alert was given by the BACTEC FX40, it was treated as a positive blood culture and processed in a manner similar to conventional blood culture system by subculturing. If there was no alert, audible or visible, the bottles were incubated for 7 days before undergoing a terminal subculture, to report as negative.

After being positive blood culture bottle whether it was BACTEC bottle or conventional (used for aerobic culture) blood culture bottle, firstly gram stain was prepared and reported to physician for empirical treatment or preliminary diagnosis. After that subculture were made on 5% sheep blood agar (BA), MacConkey's agar and Chocolate agar. The inoculated media was incubated under aerobic conditions at 37^{0}C for 24- 48 hours and read the following day.

In automated system after culture positive, identification and AST was done by VITEK-2 system.

VITEK-2 system is an automated system which automatically performs all of the steps required for identification after preparation and standardization a primary inoculum.[11] This system allows kinetic analysis by reading each test every 15 min. The optical system combines multichannel fluorimeter and photometer readings to record fluorescence, turbidity and colorimetric signals.

In conventional method, after culture positive identification was done by using various biochemical reactions and AST was done manualy on MHA using Kirby-Bauer disk diffusion method as per CLSI 2020 guidelines.

RESULTS

Table 1: Distribution Of Cultural Isolates Among Cases (n=100)

Blood culture	Automated system		Conventional system		Z test for two proportion	P value
	No Isolated	%	No Isolated	%		
Growth Negative	54	54	76	76	3.2615	0.00112
Growth Positive	46	46	24	24		
Total	100(100%)		100(100%)			

Out of 100 blood samples 46(46%) were positive by automated BACTEC system while only 24(24%) were positive by conventional blood culture. Difference in these two proportions was found statistically significantly (p value <0.05).

Table 2: Gram Positive vs Gram negative isolates.

System	Gram Negative Isolates	Gram Positive Isolates
BACTEC	36(78.26%)	10(21.74%)
Convectional	20(83.33%)	4(16.66%)

Out of 100 cases of clinically suspected septicemia, blood culture positive cases were 46 by BACTEC, of which 10 (21.74%) were gram positive isolates while 36 (78.26%) were gram negative isolates. Conventional blood culture isolation were 24 isolates of which 20(83.33%) gram negative and 4 (16.66%) gram positive organisms were found. -

Table 3: Distribution of organisms isolated by Automated System BD BACTEC Fx40.

Type	Culture Isolate	Number isolated (%)	Time Detection(h) Median (Mean)	Range of detection (h)
Gram Positive	S. aureus	4 (8.69%)	07.24 (08.25)	01.31 – 17.20
	S. epidermidis	1 (2.18%)	26	-
	S. warneri	1 (2.18%)	16.47	-
	S. capitis	1 (2.18%)	09.08	-
	S. haemolyticus	1 (2.18%)	01.07	-
	S. sciuri	1 (2.18%)	18.20	-
	Enterococcus faecalis	1 (2.18%)	28	-
Gram Negative	Acinetobacter baumannii	11 (23.9%)	05.22 (07.12)	3.23-18.46
	Klebsiella pneumoniae spppneumoniae	11 (23.9%)	7.25 (9.71)	02.18-32.00
	E. coli	7 (15.22%)	09.4 (10.07)	-
	Salmonella Typhi	2 (4.35%)	17.08 (17.08)	14.51-19.26
	Enterobacter cloacae complex	2 (4.35%)	5.56 (5.56)	04.55-06.58
	Serratia marcescens	1 (2.18%)	05.06	-
	Acinetobacter baumanni complex	1 (2.18%)	10.52	-
	Acinetobacter lwoffii	1 (2.18%)	10.52	-
Total		46 (100%)		

Table 4: Distribution of organisms isolated by conventional method.

Bacteria isolated in Conventional method	Number isolated (%)	Day of detection (D)
S. aureus	3 (12.5)	D3(2), D1 (1)
S. epidermidis	1(4.16)	D5
E. coli	5(20.84)	D3
E. cloacae	1 (4.16)	D3
Salmonella Typhi	1(4.16)	D3
Acinetobacter baumannii	7(29.17)	D1(3), D3(4)
Klebsiella pneumoniae	6(25)	D1(1), D3
Total(n=100)	24(100)	

In automated blood culture, *Acinetobacter baumannii* and *Klebsiella pneumoniae spp. pneumoniae* were the commonest isolate 11 (23.91%) and 11(23.91) followed by *E. Coli* 7 (15.22%), *CONS* 5 (10.86%), *S. aureus* 4 (8.69%), *Enterobacter cloacae complex* 2 (5.56%), *Salmonella Typhi* 2 (2.18%), *Acinetobacter lwoffii* 1 (2.18%) and *Serratia* marcescens 1 (2.18%), *Enterococcus faecalis* 1 (2.18%). The frequency distribution of various organisms isolated by conventional blood culture shows *Acinetobacter baumannii* as the commonest isolate 7 (29.17%) followed by *Klebsiella pneumoniae* 6 (25%), *E. coli* 5 (20.84%), *S. aureus* 3 (12.5%), *E. cloacae* 1(4.16%), *S. Typhi* 1 (4.16%) and *S. epidermidis* 1 (4.16%).

Table 5: Comparison of ID & AST time by automated and conventional method.

Organism	VITEK ID Mean Time(h)	VITEK AST Mean Time(h)	Conventional method ID + AST(h)
Acinetobacter baumannii	5.095	9.892	5+24 H
Acinetobacter baumannii complex	4.85	7.28	-
Acinetobacter lwoffii	8.27	13.15	-
Escherichia coli	5.135	9.884	2+24 h
Enterobacter cloacae complex	4.647	8.632	-
Enterococcus faecalis	2.7	9.78	4+24 h
Klebsiella pneumoniae spp pneumoniae	5.04	9.656	5+24 h
Salmonella Typhi	5.139	9.943	8+24 h
Serratia marcescens	3.4	16.83	-
CONS	5.161	9.754	2+24 h
S. aureus	4.958	10.04	6+24 h

For automated method, the average ID time was 4.9 h, whereas by the conventional method, it was 6 h. The mean time to final release of report(s) by the direct method was 10 h, while by standard method, it was 6 h plus 18–24 h of incubation. The shortest time for ID by VITEK was for *E. faecalis* and for AST was *Acinetobacter baumannii complex.*

DISCUSSION

Detection of septicemia and early recognition of microorganisms with

their susceptibility pattern in primary stage plays a vital role in helping the diagnosis and management of suspected cases and thus could have prognostic value.

A comparative study was carried out between conventional and automated culture system.

In our study, the bacterial isolation rate with automated blood culture was found 46% and 24% with conventional blood culture which is comparable with other studies like Azra S Hasan et al[12], Sarangi KK et al[13].

In this study, dominance of Gram-negative organisms was observed out of positive isolates by both culture systems. Similar results were found in many studies like Indian studies Mahesh et al[14], Khurana et al [15], Chandra Madhur Sharma et al[16]and IndonesiaSinto et al [17].

Acinetobacter baumannii and *Klebsiella pneumoniae* both were the predominant isolates in the current study by both methods. Comparable results were obtained in the other studies such as Khurana et al.2018[15], Mathur et al [18], Surase et al [19], Elantamilan Det al. [20] On the contrary side CONS and *S. aureus* were most common pathogen isolated in Saher, Afzal and Ansari et al.2021[21], Chandra Madhur Sharma et al. [16]

There was a significant difference in the MTTD (Mean time to detect) with BACTEC recovering all isolates within 24 h. Using BACTEC, the mean detection time was 12.18 h for all isolates as compared to 72.26 h by conventional method. The shortest time to detect by BACTEC FX40 was 43 min for *Escherichia coli.*

Other studies Kaur et al.2014[7], Gopi A, Kaushik A et al.2014[22], Gopi A, Ravikumar KL et al.2011[23], Cockerill FR et al.1997[24] have reported a mean detection time of 19–24 h hours by BACTEC and 5–7 days by conventional method.

In our study for automated method VITEK-2 compact, the average ID time was 4.9 h, whereas by the conventional method, it was 6 h. A significant difference was observed between the overall times required for the release of final report. The mean time to final release of report by the direct method was 10 h, while by standard method, it was 6 h plus 18–24 h of incubation. Similar results were obtained by Barman et al [25] and Cueto et al [26].

CONCLUSION

Despite recent advances such as PCR and other molecular techniques for microbiologic diagnosis, blood culture remains the most practical and most reliable method for detection of blood infections with a sensitivity of 35–90%.

Due to higher prevalence of MDRO and higher mortality in sepsis, antibiotics should be instituted at the earliest, as soon as the sepsis is clinically suspected. Use of automated method as BACTEC and VITEK increase sensitivity for detection as well as decrease turnaround time. Manual culture techniques usually take a longer duration for detection of infections and they are labor intensive.

To conclude, both automated method – BACTEC & VITEK together can save great time, improve quality and reduce many humanly errors or laboratory contamination in compare to conventional system which can be use in early management of critically ill patients and hasten the antibiotic therapy.

Ethics Approval: Approval for this study was obtained by order no. F.3() Acad/Ethical Clearance/2021/44 Dated 15.03.2021 from the institutional ethics committee. All participants consent were duly obtained.

Conflicts of Interest: The authors report no conflicts of interest in this work.

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