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Original Research Article

Comparative evaluation of conventional (manual) blood culture system and BacT/ALERT 3D (automated) blood culture system in a Tertiary care hospital

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Abstract: The prompt detection of bacteremia is a critical function of the clinical microbiology laboratory and blood culture remains the "gold standard" for the detection of bacteremia. Automated continuous-monitoring blood culture systems have detected growth sooner than the manual systems and they have greatly improved the efficiency of blood cultures. Here, in this hospital-based prospective study, we intended to compare such two systems with reference to yield, sensitivity and differential time to positivity. A total of 498 matched pairs of blood culture specimens were compared. Bacterial growth was identified in 183(36.74%) cultures by automated system and 146(29.31%) cultures by manual system (p=0.0153). The BacT/ALERT 3D system showed better sensitivity and specificity than manual culture system. The median time to positivity with the automated system was 15.83 hr, compared with 66.95 hr for the manual system (p<0.0001). Both systems were comparable for the recovery of majority of clinically significant isolates. The BacT/ALERT 3D automated system was very much superior to the manual culture system in the rapid detection of the organisms. The BacT/ALERT 3D ® automated microbial detection system evaluated in our study showed a marginally higher recovery rate than the conventional (manual) blood culture system used in our laboratory. However, the exceptionally faster detection rates shown by the automated system can significantly change the outcome in life threatening bloodstream infections.
Keywords: BacT/ALERT, automated blood culture, manual blood culture, time to positivity, blood stream infection

INTRODUCTION

Bloodstream infections (BSI) are major cause of morbidity and mortality. With an attributable mortality rate of around 15%, they are leading cause of death in developed as well as developing countries. The crude mortality associated with BSI ranges from 12 percent in general hospital populations to 80 percent in ICU patients. Delay in diagnosis and inappropriate empirical antimicrobial therapy are important predictors of death in patients with BSI [1].

Rapid diagnosis plays a crucial role in the final outcome of these blood stream infections and so, the prompt detection of bacteremia and fungemia is a critical function of the clinical microbiology laboratory [2]. The blood culture represents a critical tool for the detection of bloodstream infections. Despite its limitations, the blood culture remains the "gold standard" for the detection of bacteremia. An accurate interpretation of culture results is critical not only from the perspective of individual patient care but also from the standpoint of hospital epidemiology and public health [3]. Blood cultures are considered to be one of the most significant specimen types that a microbiology laboratory processes and every laboratory has a strict notification policy to ensure that positive blood cultures are promptly reported to the physician.

Manual culture techniques usually take a longer duration for detection of these infections and they are labor intensive[4]. Commercially available instrumented blood culture methods were introduced in the 1970s and they have evolved over the years. These automated continuous-monitoring blood culture systems are equipped with several features including self-contained modular incubation; agitation and detection units controlled by a computer; lack of the need for manual manipulation of culture bottles once they have

been loaded into the instrument and automated monitoring of microbial growth at 10- to 24-minute intervals [4]. Many comparative studies to date have shown that these continuous-monitoring blood culture systems have detected growth sooner than the manual systems and they have greatly improved the efficiency of blood cultures [4–6]. Here, in this study, we intended to compare the conventional blood culture system with the automated blood culture system – BacT/ALERT 3D with reference to yield, sensitivity and differential time to positivity.

MATERIALS AND METHODS

This prospective study was conducted from July 2015 to Jan 2016 (7 months) in Department of Microbiology (sample processing, isolation and detection along with antimicrobial susceptibility testing) in association with Department of Anaesthesiology (Sample collection, patient care: management and follow-up). Approval from the institutional ethics committee was duly obtained.

- Sample collection: Blood specimens were obtained at the bedside by nursing staff from wards, critical care units or by trained phlebotomist. The skin was disinfected with 2% chlorhexidine. The antecubital, median cubital fossa were the preferred sampling sites using a needle and syringe. The blood samples from the central vein catheters were obtained from needleless caps that have been disinfected with 70% isopropyl alcohol, allowed to dry and wiped with sterile gauze prior to obtaining the sample.
- Volume standards: A volume of 10 ml of blood was collected. The total volume was aliquoted into two halves (5 ml each for adults) and inoculated into an appropriate BacT/ALERT bottle and conventional blood culture bottle. To ensure that the culture bottles received in the laboratory were inoculated with the specified volume of blood, the fluid level of each container filled with blood was measured and only the blood culture bottle sets (One conventional and One BacT/ALERT bottle) which met the specified volume standards were included in the study for subsequent analyses of data.
- Processing of samples: After receiving the samples in laboratory, the bottles were checked for adequacy of volume and labelling errors. The conventional and BacT/ALERT bottles were processed accordingly as described below.

The conventional blood culture bottles employed contained Brain-Heart infusion broth (50ml for adults and 20ml for children). After collection of blood, the bottles were received and incubated aerobically at 37°C. After 18-24 hours of incubation a blind sub-culture was done to appropriate solid culture media irrespective of the turbidity status. The bottles were taken out and visually observed for turbidity every morning and then manually agitated for aeration. The bottles showing turbidity were sub-cultured appropriately. Time to positivity (time taken from sample reception to observation of turbidity) was noted for all positive samples. All the negative bottles were incubated for seven days and another blind sub-culture was done at the end of seven days of incubation before reporting them as negative.

The BacT/ALERT® 3D Microbial Detection System (BioMerieux, France) was the automated continuously monitored blood culture system used in this study. BacT/ALERT® FA plus culture bottles were used for adult patients. The bottles received were loaded into the instrument and processed according to manufacturer's instructions. The bottles flagged as positive by the instrument were unloaded and standard microbiologic procedures were followed for isolation of the organism. Time to positivity (time taken from sample reception to time flagged as positive by instrument) was noted. Though, the manufacturer's instruction was to report the bottles negative after 5 days of incubation, the incubation period was extended to 7 days for uniform comparative evaluation with the conventional blood culture system.

At the end of incubation, the bottles were marked into one of these four categories based on the results.

a) Positive: Bottles which showed turbidity or flagged positive by instrument and microorganism isolated on subculture.
b) Negative: Bottles which showed no turbidity or flagged negative by instrument after 7 days of incubation and no microorganism isolated on blind subculture at the end.
c) False positive: Bottles which showed turbidity or flagged positive by instrument, but no microorganism isolated on subcultures and no microorganism seen under direct gram stain smear examination.
d) False negative: Bottles which showed no turbidity or flagged negative by instrument after 7 days of incubation, but microorganism isolated on blind subculture at the end.

Positive cultures were reviewed appropriately and the microorganisms isolated were judged based on published criteria to be the agents of bacteremia, fungemia, contaminants and indeterminate as the cause of sepsis [3].

Data Analysis: All the relevant data were entered in Microsoft Excel (v16.0.4266) and demographic parameters were analyzed using the same. Comparison of results between conventional and automated blood culture system was done in unstratified as well as stratified manner. Stratifications were applied for individual organisms and organism

groups based on clinical importance. The comparisons between matched pairs were made using modified χ^2 test described by McNemar for paired proportions. Fisher's exact test and Pearson's Chi-Square test were applied while comparing other proportions. The yield in systems, their sensitivity and difference in time to positivity were analyzed using MedCalc ® v12.5.0 statistical software. Graphical data were generated using the same.

RESULTS

During the study period, 498 matched pairs of blood culture specimens were obtained from 353 patients (Male: Female – 1.69:1) which fulfilled the inclusion criteria for comparative evaluation. Since, the objective of the study is to evaluate the difference between matched pairs, even the paired samples collected at different sites and at different time from a same patient were considered as a separate study entity. Among these 498 sets, a total of 224 (44.98%) showed positive bacterial growth identified in either of the two culture systems. Bacterial growth was identified in 105 (21.08%) cultures by both systems, 78 (15.66%) cultures only by automated system and 41 (8.23%) cultures only by manual system. A total of 241 isolates were detected in these matched pairs; 205 were classified as clinically significant pathogens, 12 were classified as probable contaminants and 24 were classified as category unknown. In 105 cultures, where both systems showed a positive result, 91 (86.67%) detected the same organism. In 14 (13.33%) instances, both systems showed a positive result, but different organisms were isolated from the subcultures. Of the clinically significant isolates (n=205), 91 (44.39%) were recovered from both the systems, 66 (32.19%) were recovered only from BacT/ALERT 3D system and 48 (23.41%) were recovered only from Manual culture system.

In overall recovery rate, BacT/ALERT 3D system showed superiority (p=0.001) over manual culture system. The sensitivity, specificity and other diagnostic parameters of the two culture systems under study are presented in Table 1 and Fig 1. The recovery rate of members of Enterobacteriaceae was higher in BacT/ALERT 3D system (p=0.0446) when compared with manual system. But, there was no difference in the recovery rate of other clinically significant organisms like non-fermenting gram negative bacilli (p=0.1120) and gram positive cocci (p=0.8676). Interestingly, the recovery of organisms considered as probable contaminants (aerobic spore bearing bacilli and diphtheroids) was also significantly higher (p=0.0005) in the BacT/ALERT 3D culture system. Among the instances where the two systems showed discordant results, members of the family Enterobacteriaceae (p=0.0183) where isolated more frequently in BacT/ALERT system than manual system and there is no statistical difference between the two systems in detecting the gram positive cocci and non-fermenting gram negative bacilli. Comparative yields of the isolates obtained from BacT/ALERT 3D and manual culture system were presented in Table 2.

The average time to detection (TTD) was compared between the two systems and BacT/ALERT 3D system exhibited very significantly (p<0.0001) shorter time to detection. Comparison of TTD for individual organisms showed that BacT/ALERT 3D exhibited significantly shorter TTD except for miscellaneous non-fermenters (i.e Non-fermenting GNB other than *Pseudomonas* spp. and *Acinetobacter baumanii* complex) where the mean time to detection was similar (p=0.5939) between the two systems. The mean TTD for different organisms in both the systems are presented in Table 3 and cumulative frequency of detected organisms with respect to incubation period (time to positivity) is presented in Figure 2.

Table 1: Comparison of diagnostic parameters between BacT/ALERT 3D automated blood culture system and conventional (manual) culture system

	BacT/ALERT 3D automated culture system	Conventional (Manual) culture system
Sensitivity	81.7 (76-86.5)	65.18 (58.5-71.4)
Specificity	94.53 (91.1-96.9)	91.61 (87.7-94.6)
(+) likelihood ratio	14.92 (9.1-24.5)	7.76 (5.2-11.6)
(-) likelihood ratio	0.19 (0.1-0.3)	0.38 (0.3-0.5)
Youden Index	0.7622	0.5678
AUC*	0.8811 (0.8493-0.9082)	0.7839 (0.7451-0.8192)
Significance level	p<0.0001	p<0.0001

*Area under the Curve (in ROC Curve); 95% Confidence interval in brackets

Table 2 : Comparison of recovery rates of different organisms from automated and manual blood culture system

	Both BacT/ALERT 3D & Manual	BacT/ALERT 3D only	Manual only	Sig.* (p value)	Sig.* (p value)
Acinetobacter baumanii	9	18	21	0.6777[a]	<0.0001[e]
Aerobic spore bearing bacilli	0	6	0	0.0039 [b]	N/A
Candida albicans	0	3	0	0.1025 [a]	N/A
Candida spp.	0	3	9	0.0412 [c]	0.005 [e]
CONS	0	9	0	0.0002 [b]	N/A
Corynebacterium spp.	0	6	0	0.0039 [b]	N/A
Enterobacter spp.	7	15	2	0.0003 [b]	0.13 [d]
Enterococcus spp.	0	0	1	1 [a]	N/A
Escherichia coli	6	3	0	0.2064 [a]	N/A
Klebsiella pneumoniae	41	3	6	0.4846 [a]	1 [d]
NFGNB	7	4	0	0.0973 [a]	N/A
Pseudomonas aeruginosa	4	7	1	0.0304 [b]	0.417 [d]
Pseudomonas spp.	9	6	0	0.0225 [b]	N/A
Staphylococcus aureus (MRSA)	4	3	4	1 [a]	0.236 [d]
Staphylococcus aureus (MSSA)	4	7	13	0.1432 [a]	0.001 [e]
Total	**62**	**117**	**87**	**0.0503**	**.105[d]**

*Significance (p-value) calculated using Fisher's exact test / Pearson's Chi-Square test (with continuity correction)

[a] No significant difference in the recovery rate between the two systems

[b] Significantly higher recovery rate with BacT\ALERT 3D system

[c] Significantly higher recovery rate with Manual culture system

[d] No significant discordance between the results obtained with the two systems

[e] Significant discordance between the results obtained with the two systems

CONS – Coagulase negative *Staphylococcus* spp.; NFGNB – Non-fermenting gram negative bacilli other than *Acinetobacter baumanii* complex and *Pseudomonas* spp.; MSSA – Methicillin sensitive *S. aureus* and MRSA – Methicillin resistant *S. aureus*

Table 3 : Comparison of average time to detection (Time to positivity) for different organisms between the two culture systems

Organism	BacT/ALERT 3D*	Manual*	Significance[#]
Acinetobacter baumanii	20.95 (±22.29)	61.65 (±51.87)	0.0003 (Sig.)
Aerobic spore bearing bacilli	31.08 (±14.32)	N/A	N/A
CONS	20.4 (±22.36)	N/A	N/A
Corynebacterium spp.	25.92 (±18.14)	N/A	N/A
Enterobacter spp.	8.95 (±7.44)	126.81 (±50.14)	<0.0001 (Sig.)
Escherichia coli	11.64 (±0.92)	48 (±0)	<0.0001 (Sig.)
Klebsiella pneumoniae	7.76 (±5.09)	55.5 (±33.7)	<0.0001 (Sig.)
NFGNB	30.3 (±25.38)	36.14 (±12.31)	0.5939 (Not sig.)
Pseudomonas aeruginosa	24.6 (±4.24)	46 (±0)	<0.0001 (Sig.)
Pseudomonas spp.	18.36 (±7.01)	76 (±48.37)	0.0006 (Sig.)
Staphylococcus aureus (MRSA)	6.72 (±0.95)	57.5 (±36.14)	0.0009 (Sig.)
Staphylococcus aureus (MSSA)	9.92 (±4.72)	76.75 (±44.2)	0.0001 (Sig.)
Total	15.83 (±15.12)	66.95 (±44.2)	<0.0001 (Sig.)
Range	1.92 to 80.4	18.5-166.5	

***** Expressed as hours: mean (±S. D); [#]Statistical significance (p-value)

(CONS – Coagulase negative *Staphylococcus* spp.; NFGNB – Non-fermenting gram negative bacilli other than *Acinetobacter baumanii* complex and *Pseudomonas* spp.; MSSA – Methicillin sensitive *S. aureus* and MRSA – Methicillin resistant *S. aureus*)

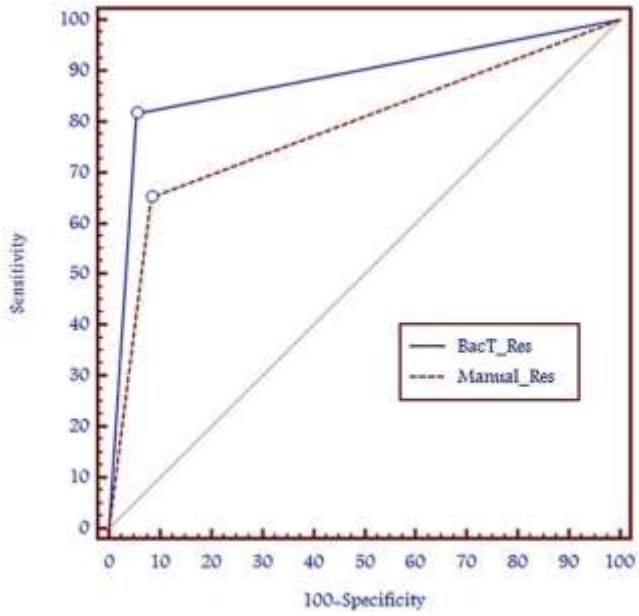

Fig 1: Comparison of the ROC curves : BacT/ALERT 3D system and Manual culture system

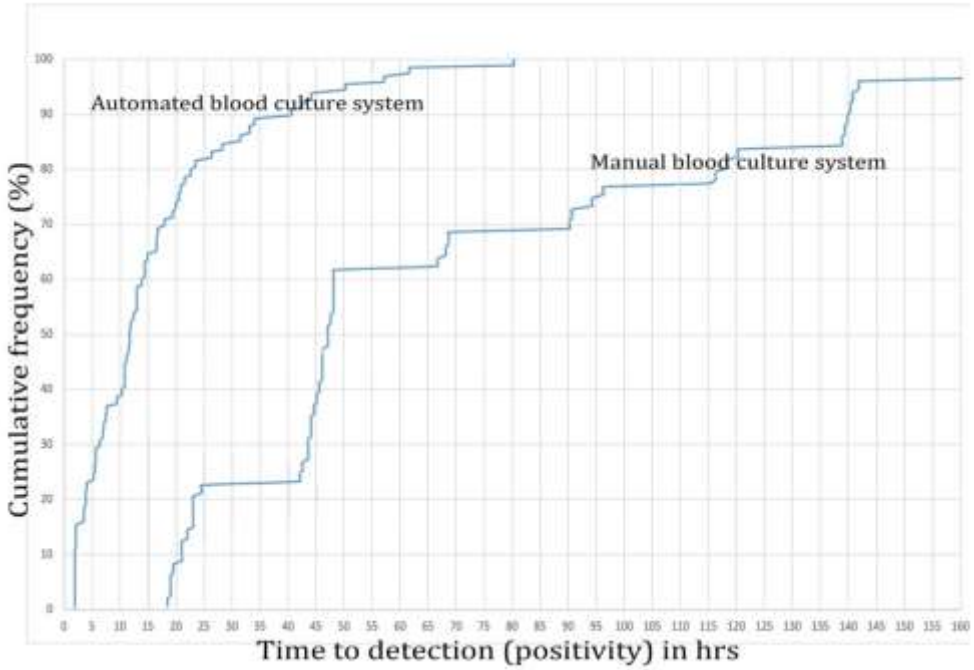

Fig 2 : Cumulative frequency distribution of detected organisms based on time to detection (positivity) in hours.

DISCUSSION

Prompt and appropriate detection of the blood stream infections is a vital function of a clinical microbiology laboratory, which in turn influences the outcome of health care in these critical conditions. The selection of a blood culture system that can provide reliable and rapid results is very essential. In the present study, the recovery rate and time to detection of microorganisms by BacT/ALERT 3D culture system and manual culture system were evaluated from our patient population.

Both systems were comparable for the recovery of majority of clinically significant isolates. The BacT/ALERT 3D automated system was very much superior to the manual culture system in the rapid detection of the organisms and even the recovery rates for commonly isolated organism groups were higher than the manual system. Many studies have reported higher recovery rate for all group of organisms when using an automated continuously monitoring blood culture system (CMBCS), [5,7,8] but, in our study there

was no difference between the two systems in the recovery rate (yield) for organisms like *Staphylococcus aureus, Escherichia coli, Enterobacter* spp. and *Acinetobacter baumanii* complex. Paradoxically, the manual culture system showed higher recovery rate for *Klebsiella pneumoniae*. However, the overall recovery rate was higher with BacT/ALERT 3D system when compared with the manual culture system.

The contamination rates have been found to be higher in different systems in different studies [5–8]. In our study, BacT/ALERT 3D automated system reported more contaminants than the manual culture system. The average time to detection has been very much shortened with the automated BacT/ALERT 3D microbial detection system used in the study. The manual culture system showed an average of 66.95 hours for positivity whereas BacT/ALERT 3D system detected much faster with an average of 15.83 hrs. In fact, the mean TTD in BacT/ALERT 3D system was shorter than the shortest TTD observed in manual system. The detection of organisms two days earlier can significantly alter the outcome in critical care settings. This finding in our study is supported by many previous studies under different conditions [7–9].

Sustained agitation and continuous monitoring of cultures for indications of growth by the BacT/Alert instrument may account for higher yields and decreased detection times for different organism groups recovered from this system. The improved recovery and time to positivity associated with agitation of culture has been documented in previous studies [10–12]. The improved yield and shorter time to detection can also be attributed to the media formulations used in these culture systems. The FA Plus bottles used in the automated culture system had added resins and antimicrobial neutralizing substances which enhanced the outcome. This has been validated in a study by Mitteregger D *et al.;* [13].

CONCLUSION

The BacT/ALERT 3D ® automated microbial detection system showed a marginally higher recovery rate than the conventional (manual) blood culture system used in our laboratory. However, the exceptionally faster detection rates in the automated system can significantly change the outcome in life threatening blood stream infections.

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