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Performance Analysis of Blood Culture by an Automated Blood Culture System at a Tertiary Care Teaching Hospital in South India

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Abstract This Prospective study analyses culture result of 134 blood culture samples received in microbiology laboratory during a five months period between October 2014 and February 2015. We have documented time required for the culture to become positive, time at which culture could be considered negative and the spectrum of isolated organismsms including their antimicrobial susceptibility patterns. The specimens were processed by using automated system BacT/Alert 3D/60. Microorganism's identification was performed by routine conventional and automated identification system and antibiotic susceptibility testing was done by Kirby bauer disk diffusion method. The mean detection time for isolates was 14.5 +/- 5.7 hours. Among organisms isolated included were Gram positive bacteria, Gram negative bacteria and yeasts with 22 (16.4%), 14 (10.4%) and 19 (14.2%) respectively. All our cultures were positive within 34 hours.

Keywords: *automated culture system, BacT/Alert 3D, turnaround time, blood culture*

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1. Introduction

Blood culture remains to be the best choice for the diagnosis of suspected bloodstream infections. Newer and rapid techniques combined with conventional methods for the isolation, identification and antimicrobial susceptibility testing would be instrumental in reducing the time required for laboratory diagnosis of septicaemia. Early confirmation of bacteraemia is crucial for better patient management which can aid clinicians to initiate appropriate antimicrobial therapy. Blood culture is considered as one of the most important clinical microbiology laboratory procedure in tertiary care centres [1,2,3,4,5].

The BacT/Alert 3D/60 (BacT) (bioMe´rieux, Inc., Durham, N.C.) blood culture system is an automated, continuous monitoring system that is widely used in clinical laboratories. This system allows rapid and accurate detection of microorganisms in blood. The BacT/Alert system uses a colorimetric sensor which measures the reflected light. The carbon dioxide (CO_2) produced and dissolved in the growth medium in the event of growth of microorganisms changes the colour of the gas permeable sensor installed at the bottom of each culture bottle from blue-green to yellow. The instrument is calibrated in such way that the light colour results in an increase of reflectance and this change is monitored and recorded by the instrument every 10 minutes. Computer algorithms determine whether sustained linear increases or increasing rates of change in CO_2 production indicate microbial growth [6,7,8].

Despite the availability of advanced and molecular techniques in diagnostic microbiology laboratories, recent studies have confirmed the usefulness of automated blood culture system as the preferred choice for diagnosis of blood stream infections. This can be attributed to a better turnaround time for the generation of results as compared to traditional blood culture and antimicrobial susceptibility testing. The main drawback include the infrastructure costs associated with automation which many developing countries can ill afford [5,9,10,11].

2. Material and Methods

This was a 5 months prospective study of all blood cultures carried out between October 2014 and February 2015 at the clinical Microbiology Laboratory of a tertiary care teaching hospital located in south India. Blood cultures were performed using the BacT/alert PF (bioMe´rieux, Inc., Durham, N.C.) culture bottles which are commercially available separately for both paediatric age group and adult patients. All culture bottles were incubated for a maximum duration of one week before being considered as negative. Cultures showing growth were processed manually for isolation and subsequent identification. Antibiotic susceptibility testing was

performed by conventional methods and automated system (Biomrieux Inc., Durham, NC, USA) where ever necessary. Serial blood cultures were reviewed, and the data was analysed for age and gender of the patients, time to positivity, microorganism type and the antibiotic susceptibility pattern.

3. Results

A total of 134 blood cultures were carried out during the period; of which, 98 (73%) were blood specimens from paediatric group while 36 (27%) from adults. There were 79 (59%) males and 55 (41%) females, in total as shown in [Table 1].

Out of 134 specimens, 56 (42%) showed organisms growth after given positive signal by BacT/alert system. Whereas 78 (58%) were reported as sterile after 7 days of incubation [Table 2]. Comparison of microorganisms isolated between the blood specimens obtained from paediatric age group and the adults is detailed in [Table 3]. Antibiotic susceptibility patterns of bacterial and fungal isolates are elaborated in [Table 4], [Table 5] and [Table 6].

Table 1. Age category and gender of patients

Age category	Male (%)	Female (%)	Total (%)
Adults	24 (18)	12 (9)	36 (27)
Children (<12 yrs)	55 (41)	43 (32)	98 (73)
Total	79 (59)	55 (41)	134 (100)

Table 2. Adults and paediatrics specimen results

Result	Adults (%)	Paediatrics (%)	Total (%)
Organisms	5 (4)	51 (38)	56 (42)
Sterile	31 (23)	47 (35)	78 (58)
Total	36 (27)	98 (73)	134(100)

Table 3. Clinical isolates recovered by system and time to detect the isolates

Organism (n)	%	Adults	Paediatrics	Mean time (hours) to detection
Staphylococcus aureus (4)	7	1	3	16.5+/-5.9
Staphylococcus epidermidis (3)	5	1	2	10+/-0.8
Micrococcus spp (15)	27	3	12	14.3+/-5.5
Acinetobacter spp (3)	5	0	3	17+/-2.5
Aerobic Spore bearing Bacilli (ASB) (1)	2	0	1	17
Citrobacter spp (1)	2	0	1	9
E coli (2)	4	0	2	12.5+/-0.5
Klebsiella spp (8)	14	0	8	12.4+/-5.5
Candida spp (19)	34	0	19	15.9+/-6.3
Total (56)	100	5	51	
Sterile (78)		31	47	

Table 4. Antibiotic susceptibility pattern of isolated Gram positive organisms

Organism / Antibiotic	*Staphylococcus aureus* (n=4) (%)	*Staphylococcus epidermidis* (n=3) (%)	*Micrococcus* spp (n=15) (%)
AK	3 (75)	2 (66)	12 (80)
AMC	1 (25)	0	8(53)
CTX	1 (25)	1 (33)	6(40)
CAZ	1 (25)	1 (33)	8(53)
CTR	1 (25)	2 (66)	8(53)
CIP	3 (75)	3 (100)	12(80)
CD	2 (50)	3 (100)	10(66)
COT	2 (50)	1 (33)	12(80)
E	1 (25)	2 (66)	10(66)
GEN	3 (75)	2 (66)	12(80)
IPM	2 (50)	2 (66)	15(100)
LZ	2 (50)	2 (66)	15(100)
OF	3 (75)	3 (100)	14(93)
OX	1 (25)	1 (33)	10(66)
T	1 (25)	0	10(66)
VAN	1 (25)	3 (100)	12(80)

Abbreviations: IPM-Imipenem, AK-Amikacin, GEN-Gentamicin, CIP-Ciprofloxacin, OF-Ofloxacin, COT-Cotimoxazole, AMC-Amoxycillin-Clavulinic acid, CAZ-Ceftazidime, CTR-Ceftriaxone, CTX-Cefotaxime, CD-Clindamycin, E-Erythromycin, LZ-Linazolid, VAN-Vancomycin, T-Tetracycline and OX-Oxacillin.

Table 5. Antibiotic susceptibility pattern of isolated Gram negative organisms

Organisms / Antibiotics	*Acinetobacter* spp (n=3) (%)	*Citrobacter* spp (n=1) (%)	*E coli* (n=2) (%)	*Klebsiella* spp (n=8) (%)
AK	2 (66)	0	2 (100)	6 (75)
AMC	0	0	0	2 (25)
CPM	2 (66)	0	2 (100)	3 (38)
CTX	2 (66)	0	2 (100)	4 (50)
CPZ	2 (66)	0	2 (100)	2 (25)
CAZ	2 (66)	0	2 (100)	3 (38)
CTR	0	0	2 (100)	3 (38)
CIP	2 (66)	0	2 (100)	6 (75)
OF	3 (100)	0	2 (100)	6 (75)
COT	1 (33)	1 (100)	0	2 (25)
GEN	2 (66)	0	2 (100)	4 (50)
IPM	3 (100)	1 (100)	2 (100)	8 (100)
PB	3 (100)	1 (100)	2 (100)	8 (100)

Abbreviations: IPM-Imipenem, AK-Amikacin, GEN-Gentamicin, CIP-Ciprofloxacin, OF-Ofloxacin, COT-Cotimoxazole, AMC-Amoxycillin-Clavulinic acid, CAZ-Ceftazidime, CTR-Ceftriaxone, CTX-Cefotaxime, CPZ-Cefoperazone, CPM-cefipime and PB- Polymixin B.

Table 6. Antibiotic susceptibility pattern of isolated yeasts

Organism / Antifungal	*Candida* spp (n=19) (%)
Amphotericin B	19 (100)
Clotrimazole	15 (79)
Fluconazole	16 (84)
Nystatin	19 (100)

4. Discussion

The results of current study revealed that there were more number of males than females, and more paediatric patients than adults investigated for bloodstream infection. [Table 1] Although the reason for the more number of paediatric patients was not quite clear, one reason could be due to the fact that it is difficult to elicit signs and clinical history in paediatric patients, thus it is essential to perform blood culture among paediatric age group to rule out septicaemia.

However, the total number of organisms isolated in paediatrics 51 (38%) were more than in adults 5 (4%) which supports the paediatrician's suspicion of septicaemia [Table 2]. This difference may be due to ingestion of antibiotics prior to blood sample collection, a situation that reduces the positivity rate in the adult population in an environment of inappropriate antibiotics use like ours. This finding is similar to other study from Nigeria [11].

The total blood cultures reported sterile were more from paediatric patients 47 (35%) as compared to adults 31 (23%) [Table 2]. Isolation of skin commensals were more from paediatrics specimens than from adults [Table 3]. Most likely factors responsible for the high rate of positive cultures among paediatric age blood cultures include, but are not limited to, poor sample collection techniques, un-trained phlebotomists and use of non standard skin antiseptics, which, unfortunately, do not include iodine or iodophore. Difficulty in collecting blood from paediatric patients also could account for the higher contamination rate. A previous study has shown that contamination rate among blood cultures can be significantly reduced by paying scrupulous attention towards aseptic skin cleansing using isopropyl alcohol and a tincture of iodine and improved venipuncture technique. The use of dedicated phlebotomists has also been found to have similar improvement effect.

In this study, overall recovery rate of cultivable microbes from paediatric blood culture was 38%. Two studies from Bangladesh and Dhaka have shown microbial isolation rates among bacteraemia as 09.88% and 14.38% respectively [12,13]. In another study from Bangladesh, the recovery rate of microbial pathogens among blood cultures was found to be 11.6% [14]. Isolation rate of 20% was reported from a study done in Nepal [15]. Slightly higher isolation rate was reported from Children's Hospital at Myanmar, where isolation rate in cases of suspected septicaemia was found as 54.2% [16]. A study of blood stream infections among children from New Delhi, India had reported the positivity of blood culture as 42% [17]. Similar study from Japan found blood culture positivity among paediatric age group as 53.6% [18].

In the present study, culture positive rate in adult patients was 4% which was contradictory to a study done by Wadud et al in 2009 which has revealed a significantly high rates of blood culture positivity in adult patients (63.51%) [13]. The disparity among various studies regarding the positivity rates of blood culture could be attributed to empirical use of broad-spectrum antibiotics before collection of blood samples.

In the current study, most of the positive cultures were obtained within 24 hours of incubation (The mean detection time for isolates was 14.5 +/- 5.7 hours.) and no positive isolates were identified beyond 2 days of incubation. The maximum incubation time of three days appears to be sufficient to consider a blood culture sample as negative. The explanation behind such observation was well supported by the outcome of a study from USA, where blood cultures had been routinely incubated for 4 days instead of the 5 days recommended by the manufacturer. It was noticed that after day 5; numerous contaminants were detected, which has led them to adopt

the policy of incubating blood culture for a maximum period of 4 days [19]. Further D J Hardy et al 1992 in their study have noted that more than 96% of cultures were positive within 2 to 3 days of incubation and have suggested to reduce the routine incubation time from 7 days to 5 or 6 days and recommended that subculture of 5 to 7 day instrument-negative BacT/Alert blood culture bottles is not necessary [8]. A study by Bourbeau P P et al concluded that 3 days of incubation was sufficient for the detection of routine bacteria and yeast from blood specimens [20].

The present study has revealed *Candida* spp 19 (34%) as the most frequent fungal isolate (Table 1).This finding is in agreement with the results of earlier studies. Early and efficient detection of yeast was noticed after using BacT/Alert when compared with conventional and other systems [5,6,7,21].

Staphylococcus aureus, Coagulase negative *Staphylococci* (CoNS), *Micrococcus* spp were isolated in this study, similar to studies elsewhere throughout the world. This suggests that not always CoNS and *Micrococcus* spp grown in blood cultures should be considered as contaminants. These organisms have been repeatedly isolated from blood cultures which have constituted to third most common cause of bacteraemia. Because of their high prevalence, especially in patients with prosthetic implants and central venous catheters, they should be considered as clinically significant.

Klebsiella species were most commonly isolated amongst gram negative bacteria followed by others like *E coli*, *Citrobacter* spp, *Acinetobacter* spp. This finding is similar with other studies [3,9,15]. Antibiotic susceptibility pattern showed that most of the bacteria were susceptible to aminoglycosides and fluoroquinolones suggesting limited use of other higher level antibiotics [Table 4 and Table 5]. This will help us to formulate antibiotic policy at hospital level.

The study revealed that among *Candida* species isolated all (100%) were susceptible to amphotericin B and nystatin. *Candida* species resistant to fluconazole and clotrimazole were observed in our study [Table 6]. The study results support the emergence of resistance among *Candida* species to most commonly used antifungal agents.

5. Conclusion

In conclusion, diagnosis of bacteraemia in tertiary care hospital settings should be carried out preferably utilizing automated techniques in order to reduce the time for the generation of results thereby improving the patient outcomes and reduce the cost associated with long hospital stays. It is also necessary to regularly evaluate the changing patterns of blood stream infections and the antibiotic sensitivity profile of isolated microorganisms. This analysis will be of great help in establishing hospital antibiotic policies. Further to this study, we recommend future prospective studies to concentrate on the possible factors responsible for the contaminations and to evaluate the impact of interventions.

Sources of Funding

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Conflict of Interest

None.

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