

# Early Identification and Antimicrobial Susceptibility Testing of Short versus Standard Incubated Blood Cultures from a Tertiary Care Centre in Southern India

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## ABSTRACT

**Introduction:** Blood cultures play an important role in the early diagnosis of sepsis and its management. Early detection of pathogens in Blood Stream Infections (BSI) and their Antimicrobial Susceptibility Testing (AST) pattern, plays a vital role in the diagnosis of sepsis and is important for guidance of appropriate therapy.

**Aim:** To evaluate the accuracy of shortly incubated blood cultures in comparison with standard method for an early Identification (ID) and AST.

**Materials and Methods:** This was a prospective observational study undertaken from July 2015 to June 2016 at Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India. The blood cultures were loaded in the BacT/Alert system. A total of 92 patients with two sets of blood cultures that flagged positive within 24 hours of collection were included in the study. Grams stain and subcultures of the broths were done. The culture plates

were examined after four hours and then at hourly intervals for the presence of growth. Once the growth was sufficient it was processed immediately for ID and AST by Vitek 2C. Incubation of the plates was continued for the rest of the 24 hours at 37°C and was processed again. The mean time for detection were compared between short and standard cultures.

**Results:** Gram negative pathogens were the predominant organisms isolated in 82/92 (89.1%) followed by gram positive in 10/92 (10.9%). The short and standard cultures had comparable results with respect to ID of the isolates. But, the AST results were comparable only in 88/92 (95.6%) patients. Of the remaining four patients, the AST showed Very Major Error (VME) in 3 (3.3%) patients and Major Error (ME) in 1 (1.08%) patient.

**Conclusion:** Short incubation of cultures enabled earliest ID and AST of the isolates from blood cultures than standard incubation.

**Keywords:** Blood stream infections, Concordance, Very major error

## INTRODUCTION

Globally, sepsis is one of the important causes of mortality [1]. Bacterial infections are the major cause of sepsis in a hospitalised patient [2]. Early detection of pathogens in BSI and their AST pattern plays a vital role in the diagnosis of sepsis and is important for guidance of appropriate therapy though the rate of positivity is low with blood cultures [3]. Immediate administration of antibiotics in sepsis is essential, so as to reduce the morbidity and mortality rate in the hospitals [4]. Early and appropriate antibiotic treatment improves the clinical outcome of patients with septicaemia; hence, there is a need to reduce the Turn Around Time (TAT) for ID and AST test results [5].

Though, automated systems like Continuous Monitoring Blood Culture Systems (CMBS) has reduced TAT, but the reporting of blood cultures takes 48-72 hours after flagging positive in a CMBS [2]. All these routine microbiological methods are time consuming, thereby delaying appropriate treatment henceforth implementation of short-term incubation method could provide ID results on the same day of blood culture positivity detection and one day earlier than the conventional AST method as this is a simple, rapid and novel method that could facilitate rapid ID and AST results on the same day and can be included into the routine workflow of clinical microbiology laboratory which could improve patient outcomes [3].

Hence, authors decided to reduce the TAT after a blood culture is flagged positive in the automated system by introducing this novel methodology in present laboratory. Present study was done to evaluate the accuracy of ID and susceptibility of the organism grown from blood cultures incubated for short time in comparison with standard method of incubation for 24 hours.

## MATERIALS AND METHODS

This was a prospective observational study undertaken at Nizam's Institute of Medical Sciences Hyderabad, Telangana, India, from July 2015 to June 2016. A total of 112 patient's blood cultures were received to the Department of Microbiology during the study period. A total of 92 patients with two sets of blood cultures that flagged positive with single organism in gram stain and culture within 24 hours of collection were included in the study. The study was an internal audit and patient identity was not revealed. However, all ethical procedures were followed.

**Inclusion criteria:** All the samples from patients with two sets of blood cultures (one set includes each one bottle of BacT/Alert SN and FAN) were included in the study.

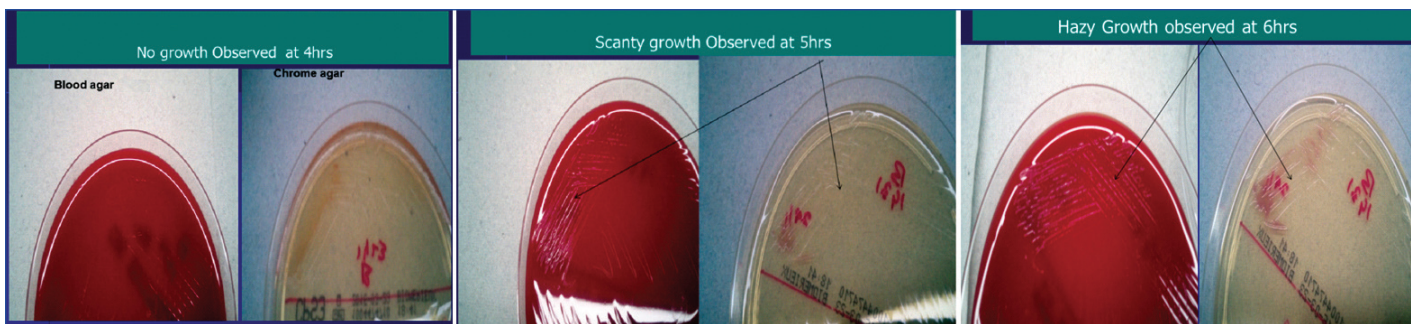
**Exclusion criteria:** The patients with one blood culture/one bottle in a set or patients with more than one type of organism on gram stain and culture were excluded from the study. Twenty patients were excluded based on the exclusion criteria.

### Study Procedure

The blood culture bottles received were loaded in the BacT/Alert system (bioMérieux, Marcy l'Etoile, France). Grams stain and subcultures of the positively flagged broths were done. The culture plates (5% sheep blood and chromogenic agar (bioMérieux, Marcy l'Etoile, France) were incubated at 37°C were examined for growth.

**Short cultures:** The cultures were examined after four hours and then at hourly intervals for the presence of growth [Table/Fig-1].

Once the growth was sufficient, it was processed immediately for ID and susceptibility (0.5 Mc Farland density) testing by



[Table/Fig-1]: Growth of the pathogens in chromogenic agar and 5% sheep blood agar at 4,5,6 hours.

Vitek 2C (bioMérieux, Marcy l’Etoile, France). The ID GN and AST N281 (bioMérieux) panels were used for detection of gram negative pathogens and ID GP and P628 panels for gram positive pathogens [3,6].

**Standard cultures:** Incubation of the plates was continued for the rest of the 24 hours at 37°C. The 24 hours cultures were again processed in the Vitek 2C for ID and AST.

**Calculation of parameters:** Mean time to flagging was calculated by dividing the sum of time taken by the blood culture bottles to flag positive by the total number of bottles flagged positive.

Mean time for appearance of growth on plates was calculated by dividing the sum of time taken for appearance of growth in positive cultures plates by the number of positive cultures.

Mean time taken for ID and AST was calculated by dividing the sum of time taken for ID and AST for each culture by the total number of positive cultures.

Total mean time to detection was calculated by adding the mean time to flagging, average time for appearance of growth on plates and average time taken for ID and AST from time of incubation.

**Very Major Error (VME):** False susceptible-If the reference result was resistant (R) and the test method result was susceptible (S).

**Major Error (ME):** False resistant-If reference result was sensitive (S) and the test method was resistant (R).

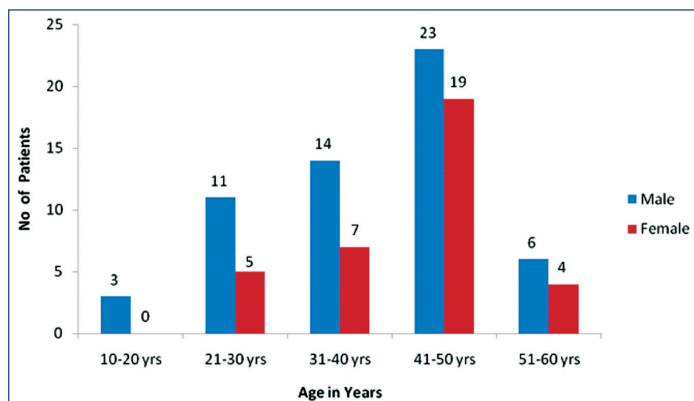
**Minor Error (mE):** If reference result was R or S and the test method result was intermediate (I) or vice versa [7-9].

### STATISTICAL ANALYSIS

In the present study, descriptive analysis was done and data was presented as percentages.

### RESULTS

Among 92 patients, 57 patients were male 35 were female. The M:F ratio is 1.6:1. Most of the patients were in the age group of 41-50 years (45.7%) [Table/Fig-2]. The clinical diagnosis of the patients is shown in [Table/Fig-3].



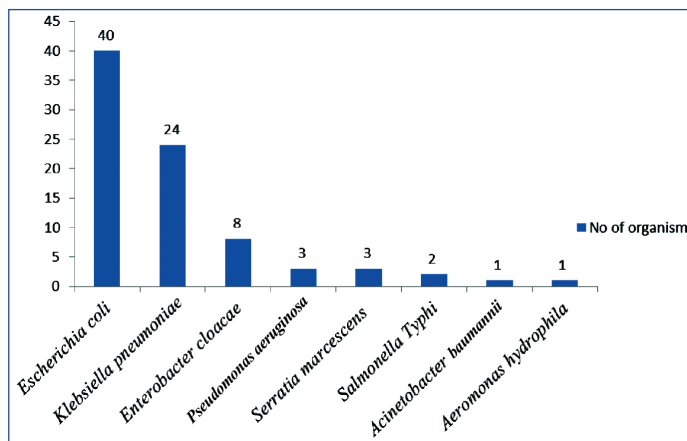
[Table/Fig-2]: Demographic data of the patients (N=92).

Gram negative pathogens were the predominant organisms isolated in 82/92 (89.1%) followed by gram positive in 10/92 (10.9%). Of the gram negative pathogens *Escherichia coli* in 40/82 (48.7%) followed

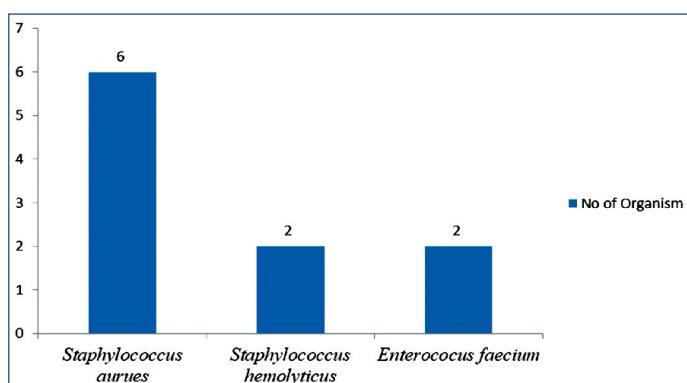
S. No.	Clinical diagnosis	No. of patients (%)
1	Abdominal surgeries	21 (22.8)
2	Surgical wound infections	17 (18.5)
3	Chronic kidney disease with sepsis	13 (14.1)
4	Urosepsis	12 (13)
5	Haematological malignancy	11 (11.9)
6	Road traffic accident with polytrauma	9 (9.8)
7	Craniotomy	3 (3.3)
8	Infective endocarditis	2 (2.2)
9	Stroke	2 (2.2)
10	Enteric fever	2 (2.2)

[Table/Fig-3]: Clinical diagnosis of patients (N=92).

by *K. pneumoniae* 24/82 (29.2%) [Table/Fig-4]. A 38/40 (95%) of *E. coli* were Extended Spectrum Beta Lactamase Producers (ESBL) and 2/40 (5%) were Multidrug Resistant (MDR) by standard culture method. A 23/24 (95.8%) of *K. pneumoniae* were ESBL producers and 1/24 (4.2%) was MDR. Of the gram positive pathogens *Staphylococcus aureus* 6/10 (60%) was the predominant pathogen isolated [Table/Fig-5].



[Table/Fig-4]: Spectrum of gram negative bacilli (n=82).



[Table/Fig-5]: Spectrum of gram positive cocci (n=10).

The short and standard cultures had comparable results with respect to ID of the isolates [Table/Fig-6]. But, the AST results

were comparable only in 88 (95.6%) patients. Of the remaining four patients, the AST showed VME in 3 (3.3%) patients and ME in 1 (1.08%) patient. The standard method identified three ESBL producers of *K. pneumoniae* which were identified as MDR by short cultures and one MDR of *E. coli* which was identified as ESBL by short cultures [Table/Fig-6].

Several studies were done to reduce the TAT for reporting of blood cultures [3,6,8,11]. Molecular studies helps in direct detection of organisms and resistance genes from blood culture broths, but it does not provide the Minimum Inhibitory Concentration (MIC) of each of antibiotic and also is not cost effective and identifies the organisms in 81% of cases [7,12,13].

Pathogens	Identification		Antimicrobial susceptibility			Discrepant result
	Short cultures	Standard cultures	Phenotype	Short cultures	Standard cultures	
<i>Escherichia coli</i>	40	40	ESBL	39	38	1 (MDR)
			MDR	1	2	
<i>Klebsiella pneumoniae</i>	24	24	ESBL	20	23	3 (ESBL)
			MDR	4	1	
<i>Enterobacter cloacae</i>	8	8	-	8	8	Nil
<i>Salmonella typhi</i>	2	2	-	2	2	Nil
<i>Serratia marcescens</i>	3	3	-	3	3	Nil
<i>Pseudomonas aeruginosa</i>	3	3	-	3	3	Nil
<i>Acinetobacter baumannii</i>	1	1	-	1	1	Nil
<i>Aeromonas hydrophila</i>	1	1	-	1	1	Nil
<i>Staphylococcus aureus</i>	6 MRSA (2) MSSA (4)	6 MRSA (2) MSSA (4)	-	6	6	Nil
<i>Staphylococcus haemolyticus</i>	2	2	-	2	2	Nil
<i>Enterococcus faecium</i>	2	2	-	2	2	Nil

**[Table/Fig-6]:** Identification (ID) and Antibiotic susceptibility Testing (AST) by short vs standard protocol blood cultures, (N=92).

ESBL: Extended spectrum beta lactamase producers; MDR: Multidrug resistant; MRSA: Methicillin resistant *Staphylococcus aureus*; MSSA: Methicillin sensitive *Staphylococcus aureus*

The mean time for sufficient growth to appear for processing was 6.6 hours and seven hours for gram negative bacilli and gram positive cocci respectively. The difference in the total mean time to detect between short and standard cultures was about 23-24 hours [Table/Fig-7].

Category	Gram negative bacilli		Gram positive cocci	
	Short culture	Standard culture	Short culture	Standard culture
Mean time to flagging (hrs)	9.6	9.6	14.4	14.4
Mean time for appearance of growth on plates (hrs)	6.6	24.0	7.0	24.0
Mean time for ID and AST from time of incubation (hrs)	12.4	18.0	12.4	18.0
Total mean time to detect	27 hrs 26 min	51 hrs 10 min	33 hrs 12 min	56 hrs 6 min
Difference in mean time	24 hrs 10 min		23 hrs 30 min	

**[Table/Fig-7]:** Mean time to detect gram negative rods and gram positive cocci by short vs standard protocol for blood cultures.

ID: Identification; AST: Antibiotic susceptibility testing

## DISCUSSION

Timely ID and providing appropriate therapy plays a crucial role in preventing the mortality associated with bacteremia [10]. Rapid ID of the organisms along with its AST results would help to improve the clinical outcome of patients [11]. Delay in reporting of AST results leads to empiric therapy with broad spectrum antibiotics and antimicrobial resistance [8]. Early report of microscopy led to the start of empiric therapy in 14.8% which was changed in 4% of patients after rapid species ID. Similarly in patients who were on empiric therapy before sending blood cultures, there was change in 6.6% after microscopy and 19.7% after species reporting the therapy changes was rational in 72.2% of the patients [6].

Identification and susceptibility testing was done directly from the positive flagged bottles or from after the growth of the organisms by short/standard incubation [3,8,11,14]. In present study, ID and AST were done after growth of cultures. Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) helps in the rapid ID of the organisms but sensitivity of the organism has to be done separately by using other methods like Vitek 2C [15]. Several methods like lysis filtration, protein extraction were studied for the direct ID of the pathogens which provided 60-99% ID of the pathogens [3,11].

In present study, the initial steps of extraction were not done and the positively flagged bottles were inoculated on chromogenic and blood agar plates and were checked hourly for sufficient growth for testing. By this method, authors tried to standardise the shortest time of incubation in comparison to the standard time of 24 hours for doing ID and AST of the pathogens. For gram negative pathogens it was around 6.6 hours and for gram positive cocci it was 7 hours.

Direct inoculation from positive blood culture bottles into Vitek 2C, 93-95% were correctly identified, but in a study from Spain 62% showed complete agreement with the standard method for species ID, while none of the gram positive cocci were correctly identified by the direct method [14,16,17]. In a study from Ireland, ID of the organisms after six hours was done by MALDI-TOF and correlation was seen in all gram positive cocci but only in 96% of gram negative bacilli [18]. Prolongation of incubation condition to six hours gives more valid results compared to shorter periods. In a study from Korea, after short term incubation for six hours they compared three methods-MicroFlex LT, Vitek-MS, and Vitek 2 Systems for ID and found that the concordance of the species level ID results obtained using MicroFlex LT and Vitek-MS with Vitek 2 system was 82.3% and 78.3% respectively [3]. In various studies they found that after three to four hours of incubation, the concordance of ID was 70-80% [19,20]. In another study 94% of the isolates were identified on the same day after standard incubation by Vitek 2 MS [11]. Overall, present study was different from most studies as they identified the

S. No.	Author	Year	Place	Method used	Errors-species level ID	Errors in AST		
						Very ME	ME	mE
1	Ha J et al., [3]	2018	Korea	Positive blood culture samples were incubated on blood agar plates for six hours and identified by the MicroFlex LT (Bruker Daltonics) and Vitek-MS (bioMérieux) systems, followed by AST using the Vitek2 System (bioMérieux).	ID rates were 82.3% and 78.3% for the MicroFlex LT and Vitek-MS platforms, respectively.	0.5%	0.7%	1.0%
2	Barman P et al., [9]	2018	New Delhi, India	Positive BACTEC blood culture bottles with monomicrobial gram negative organisms on microscopy were tested in parallel by direct ID/AST as well as conventional method.	Only one was misidentified by the direct method and there was no unidentified isolate.	Nil	0.44%	Nil
3	Machen A et al., [11]	2014	Atlanta, Georgia, United States of America.	Same day Identification (ID) and full panel antimicrobial susceptibility testing of bacteria from positive blood culture bottles by combined lysis-filtration method with MALDI-TOF Vitek mass spectrometry and the Vitek 2 system.	94.0% correct organism identification to the species level.	1.3%	1.7%	3.6%
4	Idelevich EA et al., [7]	2014	Germany	Briefly incubated agar cultures from positive blood cultures were used for AST by Vitek 2 and compared with standard cultures	-	GPC- 0.5% GNB-0 and 0.3%	GPC-0.2% GNR-0.3%	Nil
5	Munoz-Dávila MJ et al., [16]	2012	Spain.	Direct inoculation of the positive blood cultures with the Vitek cards was compared with the standard inoculation method based on the sub-culture of the positive blood culture to agar.	1.4% was misidentified and 2.8% were not identified.	0.6%	0.1%	2.1%
6	de Cueto M et al., [17]	2004	Spain	Fluid from positive blood culture bottles containing aerobic media were directly inoculated into Vitek 2 system card and compared with standard methods.	For gram negative organisms, 62% showed complete agreement while none of the 50 gram positive cocci were correctly identified by the direct method.	GNR-2.4% GPC-3.2%	GNR-0.6% GPC-2.4%	GNR-Nil GPC-2.8%
7	Present Study	2022	Hyderabad, India	Short cultures of positive flagged bottles compared with standard cultures of 24 hours.	100% correlation of identification with short and standard cultures.	3.3%	1.08%	Nil

**[Table/Fig-8]:** Comparison of similar studies from different parts of the world [3,7,9,11,16,17].

AST: Antimicrobial susceptibility testing; MALDI-TOF: Matrix-assisted laser desorption/ionization-time of flight; GPC: Gram positive cocci; GNB: Gram negative bacilli; GNR: Gram negative rod

pathogens by different instruments like Vitek, MALDI-TOF etc., after short incubation of 4-6 hours/standard incubation and same day ID. In present study, authors used Vitek 2C for ID and compared results between short and standard time incubated cultures and there was complete agreement in ID of the pathogens between six hours and 24 hours of incubation. This method gave better results than direct ID, but still has to be studied for larger number of samples.

Susceptibility testing of positive blood cultures done using the Accelerate Pheno™ system and direct Vitek® 2 card inoculation workflows gave faster and reliable results [8]. In present study, AST was done by Vitek 2C, available in present lab. The time of incubation was shortened to six hours and compared with standard time of 24 hours. In present study, there were errors in the AST in gram negative pathogens and not in gram positive pathogens. Very major error is the false susceptible result of rapid AST, ME is the false resistant result of rapid AST, and mE is the false categorisation involving intermediate result standard method and resistant or susceptible by test method [7,8]. Data showed that, for gram negative rods, there were 50% categorical agreements between the direct and standard methods for all drugs tested and 38% for gram positive cocci from direct AST from broths [17]. Comparison between direct AST from positive broths and standard AST showed VME of 3.6%, ME 2.2%, and mE 3.8% in a study from Italy [21], 0.8% VME and 0.02% ME rates from Netherlands [14], and 0.6% VME, 0.1% ME and 2.1% mE from Spain [16]. In short and standard methods of incubation, in a study from Ireland, there was 6.7% showed mE, 0.6% ME and 0.4% VME [18]. Similarly, in various other studies, VME was 0.5-1%, ME 0.7-1%, mE 1-3% [3,11]. In present study the VME was 1.08% and ME was 3.3%. VME was in one isolate of *E. coli* which was susceptible to carbapenems in short cultures and was resistant in the standard cultures. Major error in three isolates of *K. pneumoniae* which was resistant to carbapenems in short cultures and was sensitive in the standard cultures. There was agreement between other antibiotics in short and standard cultures. To our knowledge and literature search, this was the first study from Southern India.

Comparison of different studies from all over the world is shown in [Table/Fig-8] [3,7,9,11,16,17].

The US Food and Drug Administration (FDA) has set the limits for major and VME as <3% and <1.5%, respectively, and its within limits in present study [19,22]. The errors in AST could be due to the technical error while doing AST where the appropriate McFarland was not taken or due to insufficient growth of that pathogen.

In a study from United States, the average time to ID and AST from sample collection was 41.4 hours vs. 84.8 hours [11]. Another study from Spain, the mean time for ID of gram negative rods was 78.2 minutes in Brain Heart Infusion (BHI) broth for gram positive cocci was 128.5 minutes in BHI [23]. In present study, the average time to detect gram negative bacilli and gram positive cocci by short incubation was 27 hours and 33 hours respectively, in comparison to standard time which was 51 hours and 56 hours respectively. The average time difference between standard and short incubation was 23 hours and 24 hours for gram negative bacilli and gram positive cocci, respectively. Hence, there was a difference of one day between short and standard cultures, which is a very crucial period in a patient with sepsis, for initiation of antimicrobial therapy.

### Limitation(s)

The limitation of this study was that sample size was low, not evaluated for yeast isolates and is not useful in case of polymicrobial growth.

### CONCLUSION(S)

Blood cultures remain the gold standard for diagnosis of bacteremia in patients with sepsis. The time taken for reporting of blood cultures plays an important role in the management of the patients. In this study, it was observed that mean time for detection of gram negative bacteremia was lower than gram positive isolates. This simple and rapid method would facilitate the implementation of rapid ID and AST into the routine workflow of clinical microbiology laboratories, which could improve patient outcomes. With the resources available at our institute, authors observed that short incubation of cultures

helped not only in early diagnosis but also provided appropriate and prompt therapy to the patients which would prevent the mortality, length of hospital stay, cost to the patients and reduce TAT in detection of bacteremia.

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