Indian Journal of Medical Microbiology, (2012) 30(2): 187-92

Original Article

Comparative evaluation of paired blood culture (aerobic/aerobic) and single blood culture, along with clinical importance in catheter versus peripheral line at a tertiary care hospital

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Abstract

Purpose: Paired blood culture (PBC) is uncommon practice in hospitals in India, leading to delayed and inadequate diagnosis. Also contamination remains a critical determinant in hampering the definitive diagnosis. **Objectives:** To establish the need of PBC over single blood culture (SBC) along with the degree of contamination, this comparative retrospective study was initiated. **Materials and Methods**: We processed 2553 PBC and 4350 SBC in BacT/ALERT 3D (bioMerieux) between October 2010 and June 2011. The positive cultures were identified in VITEK 2 Compact (bioMerieux). True positivity and contaminants were also analyzed in 486 samples received from catheter and peripheral line. **Results**: Out of 2553 PBC samples, positivity was seen in 350 (13.70%). In 4350 SBC samples, positivity was seen in 200 samples (4.59%). In PBC true pathogens were 267 (10.45%) and contaminants were 83 (3.25%), whereas in SBC 153 (3.51%) were true positives and contaminants were 47 (1.08%). Most of the blood cultures (99.27 %) grew within 72 h and 95.8% were isolated within 48 h. In 486 PBCs received from catheter/periphery (one each), catheter positivity was found in 85 (true positives were 48, false positives 37). In peripheral samples true positives were 50 and false positives were 8. **Conclusion:** Significantly higher positive rates were seen in PBCs compared with SBCs. Automated blood culture and identification methods significantly reduced the time required for processing of samples and also facilitated yield of diverse/rare organisms. Blood culture from catheter line had higher false positives than peripheral blood culture. Thus every positive result from a catheter must be correlated with clinical findings and requires further confirmation.

Key words: *Catheter-related contamination, paired blood culture, single blood culture*

Introduction

The incidence of sepsis is increasing all over the world leading to high morbidity and mortality rates. Detection of bacteremia or fungemia by blood culture is critical in managing patients with infection, and directs the appropriate selection of antimicrobials. Blood culture is a common laboratory investigation where blood is inoculated into culture medium and continuously monitored for positive growth. Media used in blood culture bottles support the growth of most medically important bacteria and fungi, including facultative anaerobes, which grow adequately in the aerobic blood culture bottle, hence separate anaerobic bottles are infrequently used.[1,2] Manual culture techniques often take a longer duration for detection and isolation of organisms.

Contamination of blood culture samples is a frequent and economically nonviable issue in a developing country like ours. There are two schools of thought on the bacterial contamination seen in catheter-collected samples and peripheral samples. One says that bacterial contamination is higher in catheter samples, whereas others suggest vice versa.[3-6] A swift and definitive detection of bacteremia and fungemia is quintessential for improving patient care. Yet health care professionals and clinicians lack training in correct blood culture techniques.

We thus identified two objectives. First was to determine the overall positivity and time taken for detection in paired blood culture (PBC) *Vs* single blood culture (SBC). The second objective was to analyze true positives and false positives in catheter-drawn and peripheral blood culture.

Materials and Methods

Max Super Speciality Hospital is a 650-bedded hospital

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Received: 11-01-2012
Accepted: 08-03-2012

Access this article online	
Quick Response Code:	Website: www.ijmm.org
	DOI: 10.4103/0255-0857.96689

located in the Indian capital, New Delhi. There is a dedicated department of laboratory services handling 2000–2500 samples per day. Microbiology Lab is equipped with BacT/ALERT 3D blood culture system (bioMerieux, Marcy i- etoile, France) along with VITEK 2 Compact (bioMerieux, Marcy i- etoile, France) for bacterial identification and sensitivity. We used two BacT/ALERT bottles (aerobic and aerobic) for PBC in all inpatient department and outpatient department (OPD) patients above the age group of 12 years and one bottle for SBC for all pediatric patients. We have analyzed all blood cultures (paired *Vs* single) received between October 2010 and June 2011 as PBC was initiated in our institution from October 2010 onwards after the approval of the hospital ethical committee. Both bacteremia and fungemia were taken into account in all the samples processed.

Definitions

Paired blood culture

A paired culture was defined as at least one blood sample clearly labeled as drawn from a central vein catheter and at least one blood sample drawn by peripheral venipuncture.[7]

Single blood culture

A single blood culture is defined as a blood sample drawn from either from a peripheral venipuncture or a central vein catheter.[7]

True bacteremia or fungimeia

Classified paired cultures with at least one positive result as true bacteremia (or fungemia) if the following criteria were met:

(1) Certain pathogens, such as *Staphylococcus aureus*, Gram-negative bacilli (GNB) and *Candida* species isolated from any culture sample represented true bacteremia or fungemia; or (2) common skin contaminants [coagulase-negative *Staphylococci* (CoNS), *Diptheroids*, *Bacillus* species or *Micrococcus* species) or *viridans Streptococci* isolated from two or more culture samples from different sites and associated with fever (body temperature >38.3°C), rigors or hypotension (systolic BP <90 mm Hg) were considered true bacteremias. Polymicrobial infection with the same organisms in more than one culture sample was also considered to represent true bacteremia if associated with fever (body temperature >38.3°C), rigors or hypotension (systolic BP <90 mmHg).[7]

Blood culture technique

Blood samples were obtained by nursing staff from general wards or critical care units or by trained phlebotomist from OPD. Before collecting the blood sample, skin was disinfected with 2% betadine. The antecubital and mediancubital fossa were the preferred sampling sites using a sterile needle and syringe. The blood samples from central vein catheters were obtained from needleless caps that were disinfected with 70% isopropyl alcohol, allowed to dry, and wiped with a betadine pad for 30 s. The excess betadine was wiped off with sterile gauze prior to obtaining the sample. Three milliliters of blood was aspirated and discarded from both the central vein catheter and peripheral venipuncture. A new syringe was used to aspirate an additional 10 mL of blood. A blood volume of 10 mL was injected into each of two BacT/ALERT 3D blood culture bottles. All blood samples were inoculated into aerobic BacT/ALERT 3D bottles and sent to microbiology laboratory.

Sample processing: Identification and sensitivity

BacT/ALERT 3D system (bioMerieux) was used for incubation and bottles were incubated until microbial growth was detected or for five days continuously. BacT/ALERT 3D is an automated culture system, which continuously monitors for any growth in every 10 min in each bottle independently. The equipment works on the principle of colorimetry and gives a signal as soon as any trace of growth is encountered based on inbuilt set of algorithms. BacT/ALERT 3D bottles that came positive underwent gram staining and microscopy, and were plated onto sheep blood agar and MacConkey agar and further incubated at 35°C±2°C. A critical callout to the clinicians was made once the positivity was encountered and confirmed on gram staining. The positive growth was further processed for identification and sensitivity on VITEK 2 Compact (bioMerieux). VITEK 2 Compact is an automated microbial identification and antibiotic susceptibility testing system, which gives identification and antibiotic susceptibility of bacteria within 2–8 h and yeast within 4–16 h. Being a unique system it delineates the Minimum Inhibitory Concentration levels of bacteria and yeast on the commonly used antibiotics and antifungals in clinical practice. It is complemented by advanced expert system (AES), which is a powerful software giving therapeutic interpretation based on CLSI/EUCAST guidelines and phenotypic expression.

Results

We have analyzed the blood culture from 1 October 2010 to 30 June 2011. A total of 6903 blood cultures were received, of which 2553 were PBCs and 4350 were SBCs. The samples obtained were diverse, that is, from OPD, admitted patients and intensive care unit (ICU). Total blood cultures that came positive were in 550 samples; PBC were 350 (13.70%) and SBC were 200 (4.59%). In PBC true pathogens are 267 (10.45%) and contaminants were 83 (3.25%), whereas in SBC 153 (3.51%) were true positives and contaminants were 47 (1.08%).

Out of the 267 PBC true positives, GNB were 209, gram-positive cocci (GPC) were 29, yeasts were 22 and 7 cases isolated were polymicrobial. Similarly, in 153 SBC true positives, GNB were 132, GPC in 10 and

yeasts in 11. In PBC, among the GNB, *Salmonella typhi* is the most common (*n*=73) followed by *K. pneumoniae* (*n*=29), *A. baumannii* (*n*=27), *E. coli* (*n*=26) and *S. paratyphi* (*n*=24). In SBC, *S. typhi* is the most common (*n*=64) followed by *E. coli* (*n*=22), *S. paratyphi* (*n*=15), *K. pneumoniae* (*n*=12) and *P. aeruginosa* (*n*=8). *S. typhi* is the most common isolate both in PBC (*n*=73) and SBC (*n*=64), whereas CoNS is the most common isolate both in PBC (*n*=54) and SBC (*n*=31). Among the CoNS, *S. epidermids*, *S. hominis* and *S. hemolyticus* are the common contaminants on decreasing order.

Most of our blood cultures (99.27%) grew within 72 h, 95.8% were isolated within 48 h among which 75.81% were within 24–36 h of inoculation of blood [Figure 1]. The organisms which were isolated after 48 h were *S. typhi* (*n*=8), *C. glabrata* (*n*=2), *Candida* sp (*n*=1), *C. parapsilosis* (*n*=1), *E. coli* (*n*=2), *K. pneumoniae* (*n*=2), *Serratia marcescens* (*n*=1), *Trichsporon* sp (*n*=1) and *S. epidermidis* (*n*=1).

The four organisms, namely, *S. epidermidis* (*n*=2), *S. haemolyticus* (*n*=1) and CoNS (*n*=1), which were isolated after 72 h were contaminants.

Of the total 2553 PBC samples analyzed, 486 paired cultures were from catheter/periphery (one each). Catheter samples were drawn from arterial line, CentralVenousPressure line, and Hemodialysis catheter. Peripheral samples were simultaneously drawn by venipuncture. Total catheter-related true positives were 48 (9.8%) and in peripheral samples true positives were 50 (10.3%). False positives in catheter samples were 35 (7.2%) and 8 (1.6%) in peripheral samples [Figure 2]. Of the 486 cultures, the results were concordant in 435 cultures, both cultures were negative in 389 samples and in 46 paired bottles cultures were positive for the same organism. Out of the 51 discordant results, 39 were negative for peripheral line cultures but positive for catheter culture; true bacteremia was found in two cultures and the rest 37 were false positive. The remaining 12 discordant pairs tested positive for peripheral line cultures and negative for catheter; true bacteremia was found in four cultures and the rest eight were false positive.

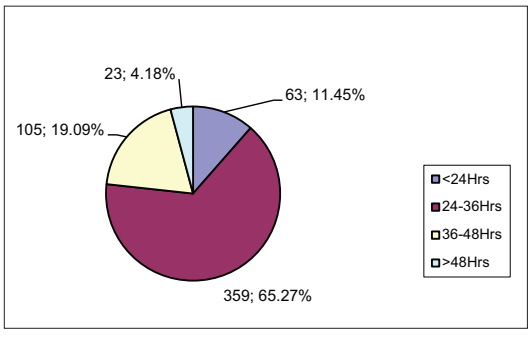

Figure 1: Time taken for positivity for all types of blood culture (both paired blood culture and single blood culture) *n*=550

True bacteremia or fungemia was 52 (10.69%) of the total 486 samples received—39 (75.0%) were gram negative, 6 (11.54%) were gram positive and 7 (13.46%) were yeasts. Among the gram negative, *K. pneumoniae* was the most common 17 (32.69%) followed by *A. baumannii* 13 (25.0%) and *P. aeruginosa* 5 (9.61%). Methicillin-resistant *S. aureus* was isolated in two cultures (3.8 %) but *Enterococcus* sp was seen in rest of the 4 (7.69%) cases. In yeast, nonalbicans *Candida* sp was 6 (11.53%) in which *C. haemulonii* was most commonly isolated 3 (5.76 %) [Table 2]. List of contaminants is mentioned in Table 3. CoNS was the most common contaminant.

Statistical analysis was performed [Table 4]. The sensitivity of catheter-drawn samples for blood culture was 92.3% and the specificity was 91.5%. The positive predictive value (PPV) of catheter-drawn samples was 56.5% and negative predictive value (NPV) was 99%. Peripheral vein (PV) drawn samples had 96.2% sensitivity and 98.2% specificity. The PPV of PV-drawn samples was 86.2% and NPV was 99.5%.

Discussion

Most laboratories in India use a single aerobic blood culture bottle for routine blood culture. Use of PBC is intended to maximize the yield of obligate aerobes, obligate anaerobes and yeast. This practice of routine use of paired aerobic and aerobic blood culture bottles were challenged in various publications, recommending using aerobic in conjugation with anaerobic bottles in order to recover significantly more organisms.[8] However, other citations noted that there is relatively a decline in the number of isolates of obligate anaerobic bacteria and concomitant increase in the number of obligate aerobic or facultative anaerobic bacteria and yeast using aerobic–aerobic PBC.[9-12] Therefore, others have recommended the use of anaerobic blood cultures, in patients with specific illness or disease or undergoing specific procedures, such as anorectal/gynaecological surgery.[9-12] Although we did not compare with anaerobic bottles, we were able to recover diverse types of significant organisms, including obligate aerobes, facultative anaerobes and yeast. The Bact/ALERT aerobic bottles (bioMerieux) not only yielded important gram-positive, such as

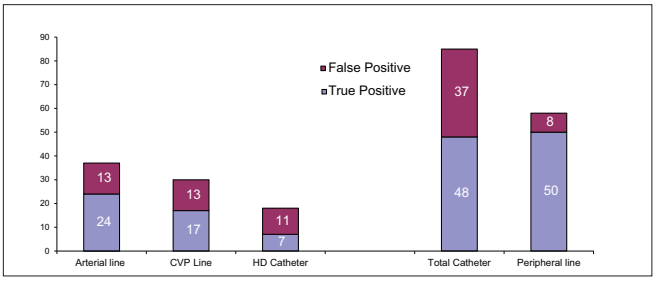

Figure 2: True positives and false positives in catheter-drawn and peripheral culture (*n*=486)

Table 1: List of true pathogens isolated from both paired and single blood cultures

	Paired (*n*=209)	Single (*n*=132)
Gram-negative bacilli		
A. baumannii	27	5
A. lwoffii	1	-
Alcaligenes fecalis	1	-
Burkholderia cepacia	1	-
Chryseobacterium idologenes	1	-
Chryseobacterium meningosepticum	2	-
E. coli	26	22
Enterobacter agglomerans	1	-
Enterobacter cloacae	2	3
K. oxytoca	1	-
K. pneumonia	29	12
P. aeruginosa	14	8
Pseudomonas putida	1	-
Salmonella typhi	73	64
Salmonella paratyphi A	24	15
Serratia marcescens	2	2
Stenotrophomonas maltophilia	3	1
Gram-positive cocci	Paired (*n*=29)	Single (*n*=10)
Enterococcus faecalis	10 (VRE 2)	1(VRE)
Enterococcus faecium	3	-
S. hominis	1	-
S. epidermidis	2	—
Strep mitis	1	—
Strep pneumoniae	4	2
S. aureus	7 (MRSA -2)	7 (MRSA -2)
S. haemolyticus	1 (MRSH)	—
Yeasts	Paired (*n*=22)	Single(*n*=11)
C. albicans	2	1
C. glabrata	2	3
C. globosa	1	—
C. haemulonii	5	—
C. intermedia	1	—
C. parapsilosis	4	1
C. tropicalis	1	2
Candida sp	4	2
Candida sake	1	—
Nonalbicans Candida sp	—	1
Trichosporon sp		1
Polymicrobial	Paired (*n*=7)	Single(*n*=0)
A. baumannii and S. aureus	1	—
A. baumannii, Candida speciesv	2	—
E. coli and E. Faecalis	1	—
E. coli, P. Aeruginosa	1	—
K. pneumoniae and A. Baumannii	1	—
P. aeruginosa, Nonalbicans Candida sp	1	—
Total	267	153

Table 1 (contd...)

VRE - Vancomycin-resistant *Enterococci*; MRSA - Methicillin-resistant *Staphylococcos aureus*; MRSH - Methicillin-resistant *Staphylococcos haemolyticus*

Table 2: Organisms isolated in catheter and peripheral line cultures

True positives	
Acinetobacter. Baumannii	13
Candida albicans	1
Candida sake	1
Candida haemulonii	3
Chryseobacterium meningosepticum	1
Candida tropicalis	1
Enterococcus faecium	3
Enterococcus faecalis	1 (VRE* 1)
Klebsiellapneumoniae	17
Nonalbicans Candida sp	1
Pseudomonasaeruginosa	5
Serratia marcescens	2
Stenotrophomonas maltophilia	1
Staphylococcus aureus	2 (MRSA-2)
Total	52

VRE - Vancomycin-resistant *Enterococci*; MRSA - Methicillin-resistant *Staphylococcos aureus*

Streptococcus pneumoniae and gram-negative bacteria, such as *Chryseobacterium meningosepticum*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia* but also led to significant recovery of rare yeasts, namely, *C. glabrata*, *C. globosa*, *C. haemulonii*, *C. intermedia*, *C. parapsilosis*, *Candida sake and Trichosporon* sp. The yield of such fungi will help the clinicians identify the yeast, which are emerging as a significant cause of blood stream infections.

CLSI guidelines states that PBC practice should be followed for adult patients suspected of bacteremia or fungemia to maximize yield of organisms.[13] In our study we have followed paired aerobic bottles for adults and SBC for pediatric patients. PBC was observed to have higher yield rates than SBC in our study. Positivity was seen in 13.70% of PBC and 4.59% positivity in our SBC samples. Thus it supports the 2004 Cockerill study, which reported the results of a similar study from 163 patients when blood cultures were performed using a continuous-monitoring blood culture system (CMBCS).[14]

We decipher that there was not an essential need of separate fungal blood culture bottle, which further necessitates requirement of additional blood sample

Table 3: List of contaminants in catheter and peripheral line cultures

CoNS	8
Acinetobacter baumannii	5
Pseudomonas aeruginosa	4
Klebsiella pneumoniae	4
Staphylcoccus. haemolyticus	3
Staphylococcus. Epidermidis	3
Candida sp	2
Enterococcus. Faecium	2
Escherichia. coli	2
Acinetobacter. baumanni and Candida sp	2
Klebsiella pneumoniae and Candida sp	1
Leuconostoc sp	1
Micrococcus sp	1
Serratia and Pseudomonas. aeruginosa	1
Candida. sake and Klebsiella.pneumoniae	1
Pseudomonas. aeruginosa and Klebsiella.pneumoniae	1
Pseudomonas. aeruginosa and Non albicans Candida sp	1
Escherichia. coli and Pseudomonas. aeruginosa	1
Trichosporon sp	1
Chryseobacterium idologenes	1
Total	45

Table 4: Diagnostic performance characteristics of blood cultures obtained through a catheter and peripheral venipuncture

Sensitivity (%)		Specificity (%)		PPV (%)		NPV (%)	
Catheter	PV	Catheter	PV	Catheter	PV	Catheter	PV
92.3	96.2	91.5	98.2	56.5	86.2	99	99.5

PV - Peripheral venipuncture; PPV - Positive predictive value; NPV - Negative predictive value

with higher cost for the culture. The results of our study supported that paired aerobic blood culture bottles will suffice the recovery of organisms including obligate aerobes, facultative anaerobes and yeast.

Early diagnosis of sepsis is important for proper management of the patient. Time to positivity of the blood culture system plays a vital role in clinching the early detection and definitive diagnosis and is a great tool for the clinicians. The speed of recovery also depends on the volume of blood drawn and time gap between the sample drawn and loading in the instrument. In our study we were able to recover most of the isolates (95.8%) within 48 h mostly due to the availability of automated blood culture system (Bact/ALERT 3D). This system also supports the recovery of most of the aerobic, facultative anaerobic and fungi significant for the clinicians' review. We also isolated diverse group of species quicker on VITEK 2 Compact, which facilitated early identification and diagnosis.

False-positive blood cultures are a constant issue for clinicians and microbiologists. The optimal strategy to minimize false positives remains a challenge, as the various results of the study have been inconsistent. False-positive culture results are costly because they often prompt more diagnostic testing and more antibiotic prescriptions with increase in hospital stay. We found that catheter-drawn blood culture had lower specificity and higher contamination (7.2%) compared with peripheral culture (1.6%). However, the true positivity was similar in both catheter (9.8%) and peripheral blood cultures (10.3%). The findings of this study were similar to Bates *et al*. and Everts *et al*. who also found higher contamination rates for catheter-drawn specimens.[15-17] Bates *et al*. and Bryant *et al*. also found that the catheter-drawn samples are more commonly contaminated than peripheral cultures. Bryant *et al*. suggested that the reason for higher contamination rate was because the mean time that the intravascular catheter was in place was 5.5 days.[18] The mean length of the line duration was not calculated in the current study but many of our contaminated cultures came from ICU patients with long-standing catheters.

Blood culture remains a valuable microbiological test for diagnosis of bacteremia and fungemia. In our study significantly higher positive rates were seen in PBC compared with SBC. Automated blood culture and identification methods significantly reduced the time required for processing of samples and also facilitated yield of gram-negative bacteria, gram-positive bacteria and yeasts including rare organisms. PBC samples drawn from separate venipuncture sites helped the clinicians to distinguish contaminants from true positive results. Blood culture from catheter line had higher false positives than peripheral blood culture. Blood culture samples drawn from intravascular devices should be avoided, or if necessary paired with a peripheral venipuncture. Thus every positive result from a catheter must be correlated with clinical findings and require further confirmation.

Acknowledgment

Authors gratefully acknowledge Dr. Navjeet Singh for technical support and senior technician Ms. Chongtham Geeta Chanu for in data management.

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How to cite this article: Tarai B, Das P, Kumar D, Budhiraja S. Comparative evaluation of paired blood culture (aerobic/aerobic) and single blood culture, along with clinical importance in catheter versus peripheral line at a tertiary care hospital. Indian J Med Microbiol 2012;30:187-92.

Source of Support: Nil, **Conflict of Interest:** None declared.