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Original Research Article

Correlation between blood volume, culture sets, and detection rates in bloodstream infections

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ABSTRACT

Background: Blood culture remains the gold standard for diagnosing bloodstream infections (BSIs), with diagnostic yield influenced by variables such as blood volume, number of culture sets, and processing time. This study evaluates these factors and their impact on blood culture positivity rates using the BD BACTEC™ FX 40 system.
Methods: A cross-sectional prospective study (June–December 2024) analysed blood cultures using BD BACTEC™ FX 40, assessing volume, sets, time to positivity (TTP), and organisms from laboratory records via standard microbiological methods.
Results: A total of 2,016 blood cultures were processed, yielding an overall positivity rate of 28.67%. Cultures with adequate blood volume (≥8 ml per bottle) demonstrated significantly higher positivity rates compared to those with suboptimal volumes ($p<0.05$). The majority of cultures (59.5%) had a volume between 10-15 ml, which also corresponded to the highest proportion of early time to positivity (TTP <24 hours). Use of two or more culture sets notably increased detection rates. Gram-positive organisms were the predominant isolates, accounting for 48.79% of positive cultures, with *Staphylococcus* spp. being the most commonly identified. Turnaround time (TAT) for final reporting varied, with the highest proportion of positive cultures (30.97%) finalized within 24-48 hours.
Conclusions: Adequate blood volume and multiple culture set significantly improve diagnostic yield in blood culture testing. Timely processing and reporting are crucial for effective clinical management of BSIs. These findings underscore the importance of adherence to recommended guidelines for blood culture collection and processing.

Keywords: Blood culture, Time to positivity, Turnaround time, Blood stream infection

INTRODUCTION

Blood cultures (BCs) are essential for diagnosing bloodstream infections (BSIs) and guiding appropriate antimicrobial therapy. BC are the test of choice to diagnose bacteraemia. Bacteraemia is defined as the presence of viable bacteria in the bloodstream, and its clinical manifestations can vary widely—from transient, asymptomatic episodes to severe systemic infections resulting in sepsis, septic shock, and death. The clinical utility of blood cultures is underscored by evidence showing that empirical antibiotic therapy is frequently suboptimal, contributing to increased morbidity, mortality, prolonged hospital stays, and elevated healthcare costs.[1,2] Various factors affect the positivity rate of BC, including the timing of sample collection, skin antiseptic preparation, number of blood culture sets, and blood volume inoculated in individual culture bottles.[3] The volume of blood collected for culture is the most significant variable influencing the microbiological detection of BSIs. It directly affects the sensitivity and diagnostic yield of blood culture systems. Evidence suggests that for each additional millilitre of blood collected, the positivity rate may increase by 2% to 4%.[4] Numerous studies have consistently demonstrated that larger blood volumes significantly enhance the likelihood

of detecting bacteraemia and fungemia, underscoring the importance of optimal sample collection practices in improving the accuracy and reliability of BC diagnostics.

With the development of BC technology, bacterial growth can be detected by automated continuous monitoring of BC systems, which is a more sensitive approach than the traditional method and shortens BC turn-around time.[5]

One pivotal study analysed 351 unimicrobial episodes where four or more BC were obtained within a 24-hour period. The findings revealed that the first BC detected 73.2% of BSIs, the first two cultures detected 93.9%, three cultures detected 96.9%, and four cultures detected 99.7% of infections. This underscores the importance of collecting multiple cultures to enhance detection rates.[6]

A previous study also revealed that 40% to 85% of the collected BC volume was inadequate.[7]

Timely identification and reporting of bloodstream infections significantly enhance patient management. A reduction in the time from BC collection to reportable results has been associated with earlier initiation of appropriate antimicrobial therapy, improved clinical outcomes, and reduced hospital expenditure.[8,9] Therefore, optimizing BC processing and minimizing turnaround time are essential components of effective antimicrobial stewardship and patient care in healthcare settings.

The aim of the present study is to evaluate the distribution of blood volumes collected in BC bottles and to analyse the impact of blood volume on the diagnostic yield, positivity rate, and time to positivity of blood cultures in patients suspected of BSIs.

In routine clinical settings, however, suboptimal blood volumes are frequently submitted, compromising the likelihood of detecting true infections and potentially delaying appropriate antimicrobial therapy. This can lead to poorer clinical outcomes, prolonged hospital stays, and increased healthcare costs.

Therefore, assessing the distribution of collected blood volumes, and analysing how it impacts positivity rates and time to positivity, is crucial. Such data can inform strategies for improving sample collection practices, increasing diagnostic efficiency, and supporting effective antimicrobial stewardship. The present study aims to address this gap and provide evidence to reinforce the importance of optimal blood volume collection in enhancing the utility of BC in the clinical diagnosis of BSIs. Optimizing preanalytical factors ensures accurate diagnosis, appropriate treatment, and better patient outcomes.

METHODS

Between May and December 2024, a cross-sectional prospective observational study was carried out at Indus International Hospital, a tertiary care centre in Punjab, India, to analyse all consecutively collected BCs. Over the course of eight months, 2016 BC bottles from 1707 patients were examined.

Blood samples collected in BD BACTEC™ FX40 blood culture bottles from patients of all age groups, whether from inpatient or outpatient departments, were included in the study. Standard aseptic techniques were followed during collection to minimize contamination and ensure the accuracy of microbial detection.

Blood samples collected in containers, vials, or blood culture bottles other than BD BACTEC™ aerobic automated BC bottles were excluded from the study. By strictly adhering to this criterion, the study focused solely on samples processed using standardized, automated methods, reducing the risk of contamination or inaccuracies associated with different collection techniques.

BC samples were processed using the BD BACTEC™ FX40 automated BC system (BD, Sparks, MD), utilizing both adult and paediatric aerobic culture bottles for incubation and detection of microbial growth.

To determine the volume of blood inoculated in each culture bottle, a pre-analytical volume estimation method was employed. A new uninoculated BD BACTEC™ FX40 BC bottles were initially weighed using a digital weighing scale to calculate the mean weight of bottle with BC broth. Subsequently, inoculated bottles received from various clinical areas were also weighed prior to incubation.

The blood volume in each bottle was calculated using the following formula: Volume (ml) = (Weight of inoculated bottle − Mean weight of empty bottles) / Density of blood (1.055 g/ml).[10] Following volume estimation, the bottles were introduced into the BD BACTEC™ FX40 instrument for incubation and continuous monitoring for growth detection.

Incubated BC bottles were monitored continuously for positive growth signals by the BD BACTEC™ FX40 system. Bottles were observed for a total of 5 days, after which cultures without positive flag in BD BACTEC™ FX40 system were reported as sterile.

BC bottles flagged as positive by the BD BACTEC™ FX40 automated system were subjected to Gram staining for preliminary identification and differentiation of microorganisms into Gram-positive, Gram-negative, or yeast. Positive samples were sub cultured onto MacConkey agar and blood agar, followed by aerobic incubation at 37°C for 16 to 18 hours. Plates were examined next day for the identification of the bacteria by standard microbiological method.

Antimicrobial susceptibility testing (AST) was performed directly from positive BC broth using the Kirby-Bauer disc

diffusion method, with results interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Parameters that were recorded were, mean volume of uninoculated BC bottles, volume of blood in each inoculated blood bottle, age distribution, gender distribution, positivity rate of BC and time to positive, microorganisms detected in cultured positive, turnaround time for final reporting. Data analysis was performed using Microsoft Excel (Microsoft Office, Redmond, WA), where descriptive statistics were applied to interpret the findings. The requirement for written informed consent was waived owing to the use of deidentified data.

RESULTS

A total of 2016 BC bottles received from 1707 patients were evaluated over a period of 6 months from June 2024 to December 2024. The volume of blood received in the BC bottles was calculated using the formula: volume = (weight of bottle filled with blood − mean weight of 50 empty bottles)/density of blood (1.055 g/ml).

A mean weight of 60.5 mL was calculated from 50 randomly selected uninoculated BD BACTEC™ FX 40 BC bottles. Upon volumetric analysis, the 10–15 ml blood volume category accounted for the majority of samples (61.25%), whereas the 20–25 ml category was the least represented (1.38%). Most BC were obtained from patients with age group (>12 years), comprising 93.78% (n=1890) of the total, with the highest number in the 10–15 mL group (n=1192). Among neonates (<28 days), 73 bottles (3.62%) were collected, with volumes ranging from 3.22 ml to 15.45 ml. For children aged 29 days to 12 years, 53 bottles (2.63%) were received, with a notable preference for the 5–10 ml volume range (n=25). Blood culture testing was performed more frequently in male patients (58.87%) than in females (41.12%) (Table 1).

Table 1: Distribution of blood culture bottles received according to the volume of blood and their distribution according to age and gender.

Volume	Total	Age			Gender	
Blood volume	**Number of blood culture bottles received**	**<28 days**	**29 days-12 yrs**	**Adults >12 yrs**	**Male**	**Female**
<5 ml	49 (2.43%)	21	1	27	30	19
5-10 ml	619 (30.70%)	37	25	567	330	289
10-15 ml	1235 (61.25%)	11	22	1192	763	472
15-20 ml	85 (4.21%)	4	3	78	49	36
20-25 ml	28 (1.38%)	0	0	28	15	13
Total	2016	73 (3.63%)	53 (2.62%)	1890 (93.78%)	1187 (58.87%)	829 (41.12%)

The majority of patients (82.77%) had only one BC bottle collected, which may limit the diagnostic sensitivity, (16.69%) had two bottles, three (0.29%), four (0.17%), or five (0.06%) bottles collected (Table 2).

Table 2: Distribution of number of blood culture bottles received.

Number of blood culture bottles received	Number of patients	Percentage (%)
1 bottle	1413	82.77
2 bottles	285	16.69
3 bottles	5	0.29
4 bottles	3	0.17
5 bottles	1	0.06

The majority of patients (82.77%) had only one BC bottle collected, resulting in a 27.88% positivity rate. As the number of bottles increased, so did the positivity rate—reaching 80.00 % in the patient with five bottles.

Patients with two bottles had a slightly higher positivity (28.19%), while those with three or more showed a marked increase (Table 3).

Figure 1 illustrates a positive correlation between the number of BC bottles collected per patient and the likelihood of culture positivity. As the number of bottles increased, the probability of detecting bloodstream infections also increased.

Of 578 positive blood cultures, the majority (59.5%) had a volume between 10–15 ml, which also yielded the highest proportion of early time to positivity (TTP) (<24 hours). Only 2.76% of positives were from <5 ml volumes, with a significantly higher proportion showing delayed positivity (>48 hours). Specifically, cultures with <5 ml volumes had the highest share of TTP >48 hours (68.75%), compared to only 53.77% in the 10–15 ml group (Table 4).

Among the culture-positive samples, Gram-positive cocci were the most frequently isolated organisms (48.79%), dominated by coagulase-negative Staphylococcus spp. (35.64%), followed by Staphylococcus aureus (9.34%) and Enterococcus spp. (3.46%). Gram-negative bacteria comprised 43.43% of isolates, with *Klebsiella* spp. (10.55%) and *Escherichia coli* (6.57%) being the predominant *Enterobacter* ales. Non-lactose fermenters, notably Pseudomonas spp. (12.97%) and Acinetobacter spp. (6.92%), were also significant. Yeast accounted for

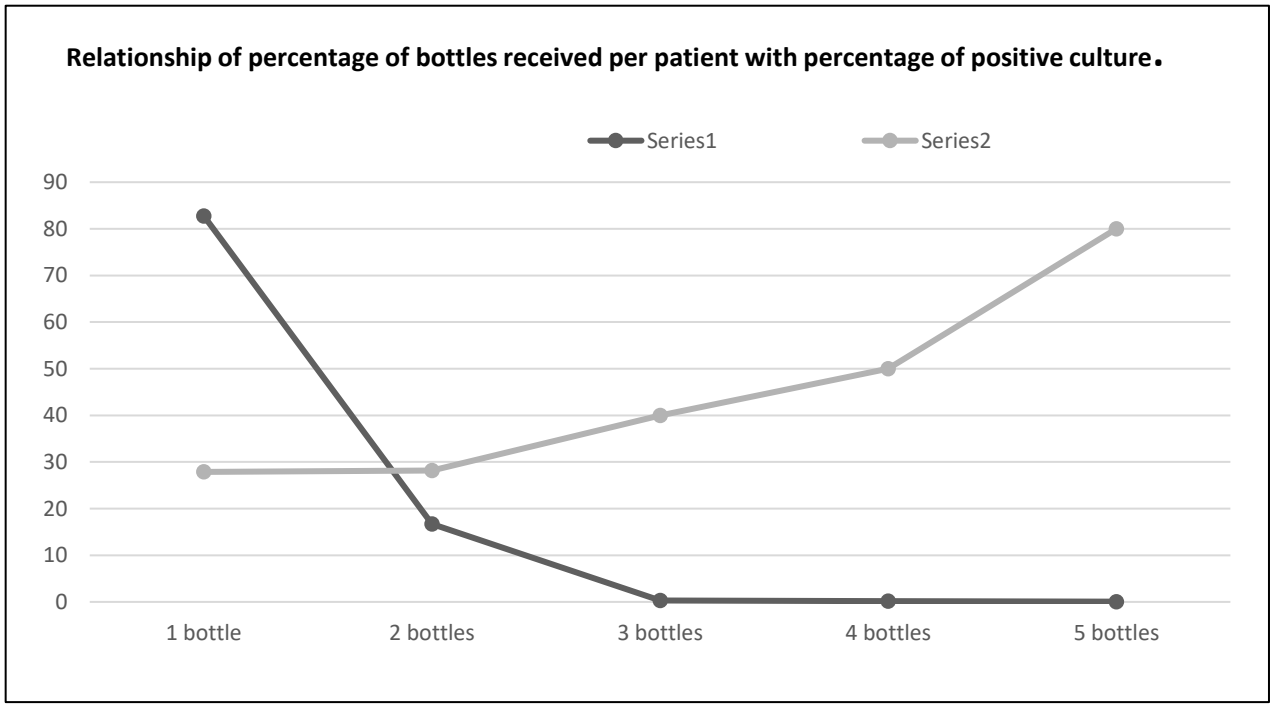
4.50% of isolates. Contaminants, such as *Diphtheroids*, *Bacillus*, and *Micrococcus* spp., made up 3.29%, indicating possible breaches in aseptic collection techniques (Table 5).

Table 3: Detection of growth in relation to blood culture set number.

Number of blood culture bottles received	Number of patients	Percentage (%)	Total number of blood culture bottles	Total number positive culture	Percentage (%) of positive culture
1 bottle	1413	82.77	1413	401	27.88
2 bottles	285	16.69	571	161	28.19
3 bottles	5	0.29	15	6	40.00
4 bottles	3	0.17	12	6	50.00
5 bottles	1	0.06	5	4	80.00
Total	1707		2016	578	28.67

Table 4: Detection of growth in relation to blood volume.

Volume (in ml) Time to positivity (TTP)	<5 ml N (%)	5-10 ml N (%)	10 -15 ml N (%)	15-20 ml N (%)	20-25 ml N (%)	Total N (%)
Total positive blood cultures	16(2.76)	181(31.31)	344(59.51)	27(4.67)	10(1.73)	578
TTP <12 hours	1(6.25)	22(12.15)	40(11.62)	3(11.11)	0	66 (11.45)
TTP 12-24 hours	2(12.50)	29(16.02)	58(16.86)	2(7.40)	0	91 (15.74)
TTP 24-48 hours	2(12.50)	20(11.04)	61(17.73)	7(29.92)	0	90 (15.57)
TTP 48-72 hours	8(50.00)	53(29.28)	97(28.19)	8(29.62)	4 (40.00)	170 (29.41)
TTP >72 hours	3(18.75)	57(31.49)	88(25.58)	7(29.92)	6(60.00)	161 (27.85)



FOOTNOTE:

Series 1- represents percentage of blood culture bottles received per patient.
Series 2- represents the percentage of blood culture bottles positive per patient received.

Figure 1: Relationship of percentage of bottles received per patient with percentage of positive culture.

Table 5: Microorganisms distribution in positive blood cultures.

	Microorganism	Number of culture positive	Percentage (%) of culture positive
Gram positive cocci	Coagulase negative staphylococcus spp.	206	35.64
	Staphylococcus aureus	54	9.34
	Enterococcus spp.	20	3.46
	Streptococcus spp.	2	0.35
Gram negative bacteria			
Enterobacterales	Klebsiella spp.	61	10.55
	Escherichia coli	38	6.57
	Enterobacter spp.	12	2.08
	Salmonella spp.	8	1.38
	Proteus spp.	5	0.86
	Citrobacter spp.	3	0.52
Non lactose fermenting bacteria	Pseudomonas spp.	75	12.97
	Acinetobacter spp.	40	6.92
	Stenotrophomonas maltophilia	9	1.55
Yeast	Yeast	26	4.50
Contaminants	(diphtheroides/ bacillus/ micrococcus)	19	3.29

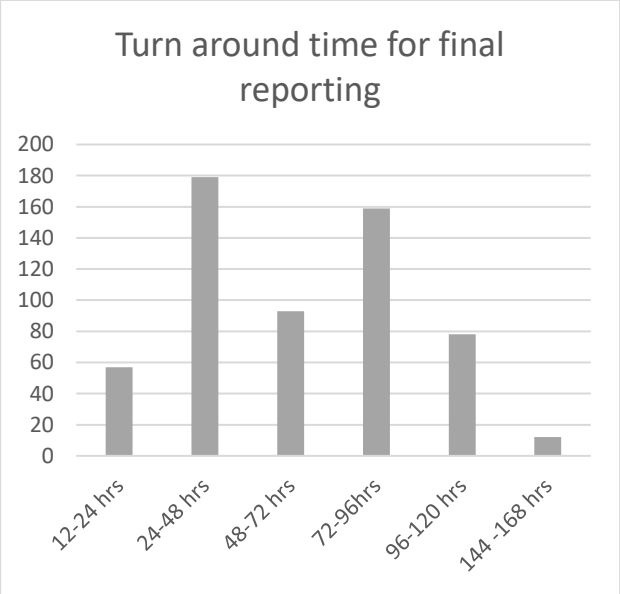


Figure 2: Turnaround time of positive blood culture final reporting starting from time of receiving sample.

The turnaround time (TAT) for final BC reporting varied significantly. The highest proportion of positive cultures (30.97%) were finalized within 24–48 hours, followed by 27.51% reported between 72–96 hours. A smaller yet substantial number (16.09%) were completed in 48–72 hours, while 13.49% were finalized within 96–120 hours. Only 9.86% of positive cultures were reported rapidly, within 12–24 hours, and 2.08% required 120–144 hours. These findings underscore the importance of early detection and reporting, with the majority of results (77%) finalized within 96 hours, supporting timely clinical decision-making in suspected bloodstream infections (Figure 2).

DISCUSSION

BC remains the gold standard for diagnosing BSIs, providing crucial information for both pathogen identification and antibiotic susceptibility. This study, conducted in a tertiary care hospital using the BD BACTEC™ FX 40 automated BC system, assessed the impact of blood volume, number of culture bottles collected, and TAT on the positivity rate and diagnostic yield of BC. Our findings support the existing body of literature highlighting the importance of proper sample collection practices and efficient laboratory workflows to optimize patient outcomes.

The total volume of blood inoculated in culture bottles is widely recognized as the single most important factor influencing the detection of bacteraemia or fungemia. Our analysis of 2016 BC bottles revealed that the 10–15 ml volume group accounted for the majority (61.25%) of samples, a finding aligned with CLSI and ASM recommendations advocating for 8–10 mL per bottle for adults.[11,12] The sensitivity of BC increases proportionally with blood volume, with an estimated 2% to 4% increase in positivity per additional millilitre of blood collected.[3-13] This association was reflected in our study: most of the positive cultures were reported in the 10–15 ml volume range, and volumes below 5 ml were associated with significantly lower detection rates. Importantly, 2.43% of our samples had <5 ml blood volume, consistent with previous reports showing inadequate collection in 40–85% of cases.[14]

Age-based distribution showed that the vast majority (93.78%) of BC were obtained from adult patients. In children, especially neonates, limited blood volume poses a challenge to adequate sample collection. However,

targeted efforts should still be made to approach the recommended paediatric volumes, considering the increased diagnostic yield with higher blood volume, even in smaller age groups.[15]

A critical finding of this study was the positive correlation between the number of BC bottles collected per patient and the rate of culture positivity. Among patients from whom only one bottle was collected (82.77%), the positivity rate was 27.88%. This rate increased to 80.00% in the single patient from whom five bottles were collected. This trend mirrors a pivotal study by Lee et al, which showed that while one BC detected 73.2% of infections, the yield increased to 99.7% when four cultures were obtained within 24 hours.[6] Multiple sets improve the sensitivity for intermittent bacteraemia and reduce the likelihood of false-negative results. However, despite this evidence, resource constraints and collection practices still limit the number of sets drawn, particularly in high-throughput or emergency settings.

The analysis of time to positivity (TTP) provided insights into the effectiveness of the BD BACTEC™ FX 40 system. Our study showed that most positive results (30.97%) were available within 24–48 hours, while a majority (77%) of all positives were finalized within 96 hours. Shorter TTP is often associated with higher bacterial loads, particularly in cases of sepsis and acute infections, and provides a window for early intervention. Cultures in the 5–10 ml and 10–15 ml ranges exhibited the fastest TTP, supporting prior observations that optimal blood volumes reduce the time required to reach a positive signal.[16]

Microbiological profiling in our study revealed that Gram-positive cocci accounted for the largest proportion of positive cultures (48.79%). Coagulase-negative Staphylococci (CONS), at 35.64%, were the most commonly isolated organisms. While often dismissed as contaminants, CONS have emerged as significant pathogens in immunocompromised individuals and those with indwelling devices.[17] The second most common Gram-positive isolate was *Staphylococcus aureus* (9.34%), a known cause of serious BSIs associated with high morbidity and mortality. Among Gram-negative organisms (43.43%), *Klebsiella spp.* (10.55%) and *Pseudomonas spp.* (12.97%) were predominant, reflecting local epidemiological trends and potential nosocomial transmission. Yeast, which constituted 4.5% of isolates, is an important cause of fungemia in ICU patients and immunocompromised hosts.

The presence of contaminants such as Diphtheroid, Bacillus spp., and Micrococcus spp. in 3.29% of cultures is consistent with other reports suggesting contamination rates of 2-3%.[4] These cases highlight the need for rigorous aseptic technique during collection, as contamination can lead to unnecessary antimicrobial therapy and prolonged hospital stays. Standardized skin antisepsis protocols and staff training are essential to minimize such occurrences. The clinical impact of BC goes beyond mere pathogen identification. Several studies have demonstrated that empirical therapy is frequently inappropriate, and delayed optimization can lead to increased morbidity, prolonged hospitalization, and higher healthcare costs.[18] In our study, only 9.86% of positive cultures were finalized within 12–24 hours, suggesting room for improvement in processing times. Reducing TAT through enhanced laboratory workflows and direct-from-blood susceptibility testing could facilitate earlier initiation of targeted therapy. Rapid diagnostic technologies, including MALDI-TOF MS and molecular assays, have been shown to significantly reduce time to pathogen identification and should be explored further in conjunction with automated culture systems.[19]

The findings of our study reinforce the critical importance of pre-analytical variables in BC diagnostics. The inadequate volume of blood, low number of sets, and delays in collection can significantly compromise the diagnostic yield. Education of healthcare providers regarding best practices in sample collection is therefore paramount. Additionally, stewardship interventions should promote the appropriate number of cultures to enhance diagnostic precision and reduce the use of broad-spectrum antimicrobials. This study provides thorough analysis of critical pre-analytical variables in BC diagnostics, with a particular focus on volume and number of bottles. The findings of the study highlight the importance of optimizing BC practices to improve diagnostic accuracy, turnaround time, and ultimately patient outcomes. Our study had certain limitations. Being conducted at a single centre may limit generalizability. Also, while we focused on volume and number of bottles, other variables such as timing of collection in relation to fever onset, prior antibiotic use, and sample handling were not assessed. Future studies incorporating these factors and evaluating patient outcomes in correlation with culture positivity and turnaround times would be beneficial.

CONCLUSION

This study highlights the strong relationship between adequate blood volume, number of culture sets, and diagnostic yield of BCs. Despite advances in automated detection, the value of these systems is heavily dependent on pre-analytical quality. Clinicians and microbiologists must work collaboratively to ensure adherence to BC best practices, thereby improving the timely detection and management of bloodstream infections.

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