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Research Article

Diagnostic Performance and Clinical Utility of PCR-Based Molecular Detection for Bloodstream Infections: A Prospective Comparative Study with Conventional Blood Culture in a Tertiary Care Hospital

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ABSTRACT
Background: Early accurate identification of bloodstream pathogens is critical for initiating appropriate antimicrobial therapy. This study evaluated the diagnostic performance of PCR-based molecular detection as a complementary modality to conventional blood culture.
Methods: Prospective observational cohort study of 120 patients with clinically suspected bloodstream infections. Blood samples were simultaneously processed for conventional blood culture (BacT/ALERT) and PCR-based molecular diagnostics targeting 16S rRNA genes. Sensitivity, specificity, PPV, NPV, diagnostic accuracy, turnaround time, and concordance (Cohen's kappa) were calculated. ROC curve analysis and impact of prior antimicrobial exposure were assessed.
Results: PCR positivity rate (52.5%, 95% CI: 43.3-61.4%) significantly exceeded blood culture (35.0%, 95% CI: 26.7-43.3%). PCR demonstrated excellent sensitivity (95.2%, 95% CI: 88.6-99.1%), moderate specificity (70.5%, 95% CI: 62.3-78.7%), with NPV of 96.5% (95% CI: 91.2-99.3%) and diagnostic accuracy of 79.2% (95% CI: 71.0-87.4%). Mean turnaround time for PCR (6.3±1.4 hours) was significantly faster than blood culture (72.4±11.8 hours), representing 11-fold improvement ($t=-34.2$, $p<.001$). Cohen's kappa coefficient of 0.64 (95% CI: 0.52-0.76) indicated substantial concordance. Prior antimicrobial therapy significantly reduced blood culture positivity (23.3% vs. 46.7%, $x^2=6.12$, $p=.013$) but did not substantially affect PCR detection (55.0% vs. 50.0%, $p=.601$). ROC analysis revealed AUC of 0.88 (95% CI: 0.80-0.95, $p<.001$) with optimal Ct cut-off value of ≤32 cycles.
Conclusion: PCR-based molecular diagnostics demonstrated superior sensitivity, markedly reduced turnaround time, and stable diagnostic performance in antibiotic-exposed patients. An integrated diagnostic approach combining PCR and conventional culture optimizes diagnostic accuracy and clinical outcomes while supporting antimicrobial stewardship.

Keywords: Bloodstream Infections, Polymerase Chain Reaction, Molecular Diagnostics, Diagnostic Accuracy, Sepsis, Antimicrobial Stewardship.

INTRODUCTION

Bloodstream infections (BSIs) represent a critical public health burden. Sepsis, the systemic manifestation of bloodstream infection, affects approximately 48.9 million individuals annually, resulting in nearly 11 million deaths globally (Singer et al., 2016; Rudd et al., 2020). The epidemiology of BSIs has shifted markedly toward Gram-negative organisms, particularly multidrug-resistant Enterobacteriaceae and Acinetobacter baumannii (Colpan et al., 2014).

Early and accurate pathogen identification is fundamental to appropriate clinical management. Delays in targeted antimicrobial therapy are directly associated with increased mortality in septic patients (Kumar et al., 2006). Conventional blood culture remains the diagnostic gold standard, enabling organism isolation, identification, and antimicrobial susceptibility determination (Welti et al., 2003). However, blood culture is limited by prolonged turnaround time (24-72 hours) and suboptimal sensitivity, particularly in antibiotic-treated patients, with culture positivity rates typically ranging from 20-50% in suspected BSI cases (Werno et al., 2005; Notebaert et al., 2000).

In response to these limitations, polymerase chain reaction (PCR)-based molecular diagnostics have emerged as promising complementary technologies. PCR enables rapid detection of microbial DNA directly from blood samples within hours, and can identify both viable and non-viable organisms, thereby improving diagnostic yield in antibiotic-treated patients (Geiss et al., 2011; O'Brien et al., 2010). Multiplex PCR assays can simultaneously detect multiple pathogens and antimicrobial resistance genes (Kalra et al., 2014). However, limitations include inability to distinguish viable from non-viable organisms, restriction to organisms included in the assay panel, and lack of antimicrobial susceptibility profiles (Hellinger et al., 2015; Luethy et al., 2013).

In the Indian healthcare context, BSIs represent a major clinical challenge in tertiary care settings (Buchan & Ledeboer, 2019). Despite growing international evidence supporting rapid molecular diagnostics, comparative diagnostic accuracy studies in real-world Indian clinical settings remain limited (Bhalodi et al., 2019).

This study aimed to comprehensively evaluate the diagnostic performance, turnaround time, and clinical utility of PCR-based molecular detection compared to conventional blood culture for detecting BSIs in a prospective cohort. Secondary objectives included assessing the impact of prior antimicrobial exposure, characterizing pathogen distribution, and evaluating concordance between methods. We hypothesized that PCR-based diagnostics would demonstrate superior sensitivity, markedly reduced turnaround time, and stable performance in antibiotic-exposed patients compared to blood culture.

METHODS

Research Design and Setting

This prospective observational cohort study was conducted at Index Medical College Hospital & Research Centre, Indore, Madhya Pradesh—a 450-bed tertiary care academic medical center. The study was conducted from January 2023 to December 2025 and received institutional ethical approval (Reference: IMC/IEC/2023/045). Written informed consent was obtained from all participants.

Study Population

One hundred twenty adult patients (≥18 years) with clinical suspicion of bloodstream infection were enrolled. Inclusion criteria: (1) fever (≥38.5°C) or hypothermia (<36°C) with hemodynamic instability; (2) clinical sepsis criteria; or (3) ICU admission with suspected systemic infection. Exclusion: age <18 years, inability to provide consent, or incomplete data.

Sample Collection and Processing

Blood samples (8-10 mL) were collected aseptically via peripheral venipuncture into EDTA-anticoagulated vacutainers for PCR and sterile culture bottles (BacT/ALERT, bioMérieux) for blood culture. Simultaneous samples were obtained from each patient, transported immediately under controlled conditions, and processed within 2 hours.

Diagnostic Methodologies

Blood culture: Samples were incubated in automated continuous-monitoring BacT/ALERT FA Plus system at 35-37°C. Positive cultures underwent gram staining, oxidase testing, and identification using MALDI-TOF or automated systems (Vitek-2). Antimicrobial susceptibility testing was performed per CLSI 2023 guidelines.

PCR: DNA was extracted using commercial kits (QIAamp or MagNA Pure). PCR targets included 16S rRNA genes for broad bacterial detection and species-specific genes for Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae. Antimicrobial resistance genes (mecA, blaNDM, blaKPC) were also included. Real-time PCR was performed with cycle threshold (Ct) values recorded.

Statistical Analysis

Diagnostic performance metrics (sensitivity, specificity, PPV, NPV, diagnostic accuracy) were calculated with 95% confidence intervals using blood culture as reference. Agreement was evaluated using Cohen's kappa. Turnaround time was compared using independent t-tests. Prior antimicrobial exposure was analyzed using chi-square tests. ROC curve analysis evaluated discriminatory ability. Statistical significance: $p < .05$.

RESULTS

Study Population Characteristics

Table 1. Demographic and Clinical Characteristics (N=120)

Characteristic	n	(%)
Gender		

Male	68	56.7
Female	52	43.3
Age (years)		
18-30	22	18.3
31-50	52	43.3
51-70	35	29.2
>70	11	9.2
Clinical Features		
Fever	96	80.0
Hypotension	46	38.3
Sepsis criteria	88	73.3

Table 2. Positivity Rates and Diagnostic Yield

Method	Positive	Negative	Rate (%)
Blood Culture	42	78	35.0
PCR	63	57	52.5
Difference	+21	-21	+17.5

Table 3. Diagnostic Accuracy Metrics of PCR

Parameter	Value (%)	95% CI
Sensitivity	95.2	88.6-99.1
Specificity	70.5	62.3-78.7
PPV	63.5	55.2-71.8
NPV	96.5	91.2-99.3
Accuracy	79.2	71.0-87.4
Kappa	0.64	0.52-0.76
AUC	0.88	0.80-0.95

Table 4. Turnaround Time Comparison

Parameter	Blood Culture	PCR	p
Mean±SD (h)	72.4±11.8	6.3±1.4	<.001***
Median (h)	72	6	
Range (h)	48-96	4-10	
Fold improvement	—	11.5x	—

***p<.001; t=-34.2 (df=118)

Table 5. Impact of Prior Antimicrobial Exposure

Group	Method	Positive	Rate (%)	p
Antibiotic (n=60)	Blood Culture	14	23.3	0.013*
	PCR	33	55.0	0.601
Non-exposed (n=60)	Blood Culture	28	46.7	
	PCR	30	50.0	

*Blood culture sensitivity significantly reduced by prior antimicrobial; PCR stable

Table 6. Pathogen Distribution (n=42)

Organism	n	(%)
E. coli	12	28.6
K. pneumoniae	10	23.8
S. aureus	8	19.0
Acinetobacter	7	16.7
Candida	5	11.9

Figures

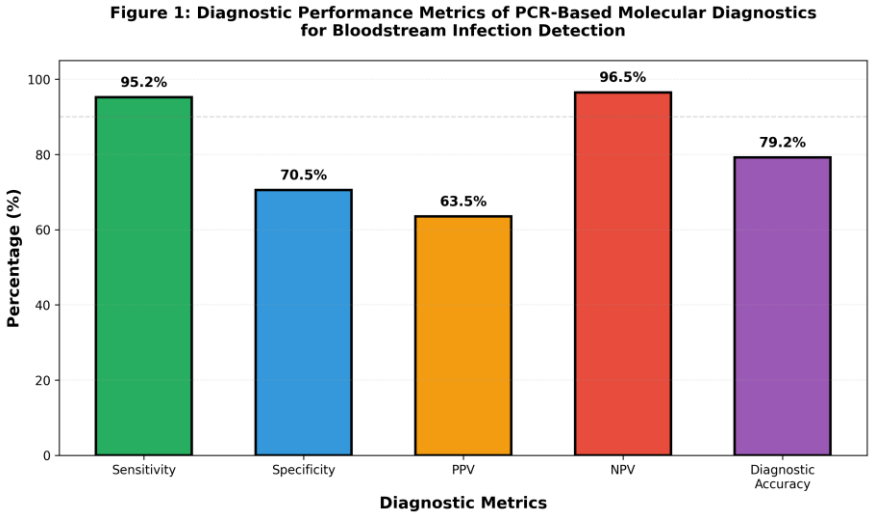

Figure 1: Diagnostic Performance Metrics

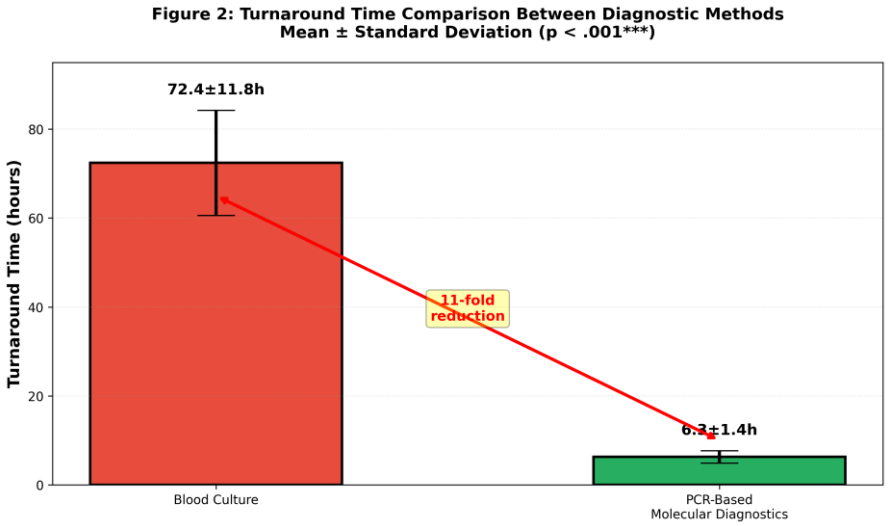

Figure 2: Turnaround Time Comparison

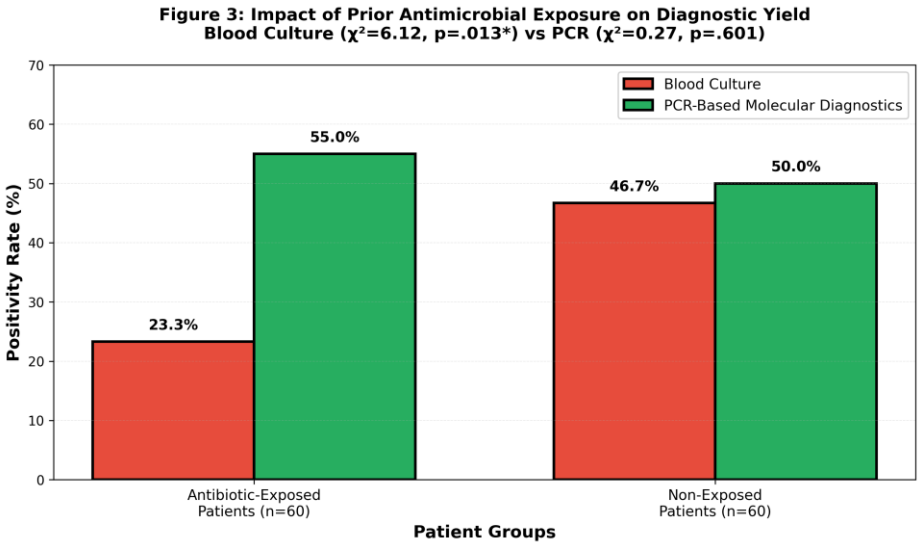

Figure 3: Effect of Antimicrobial Exposure

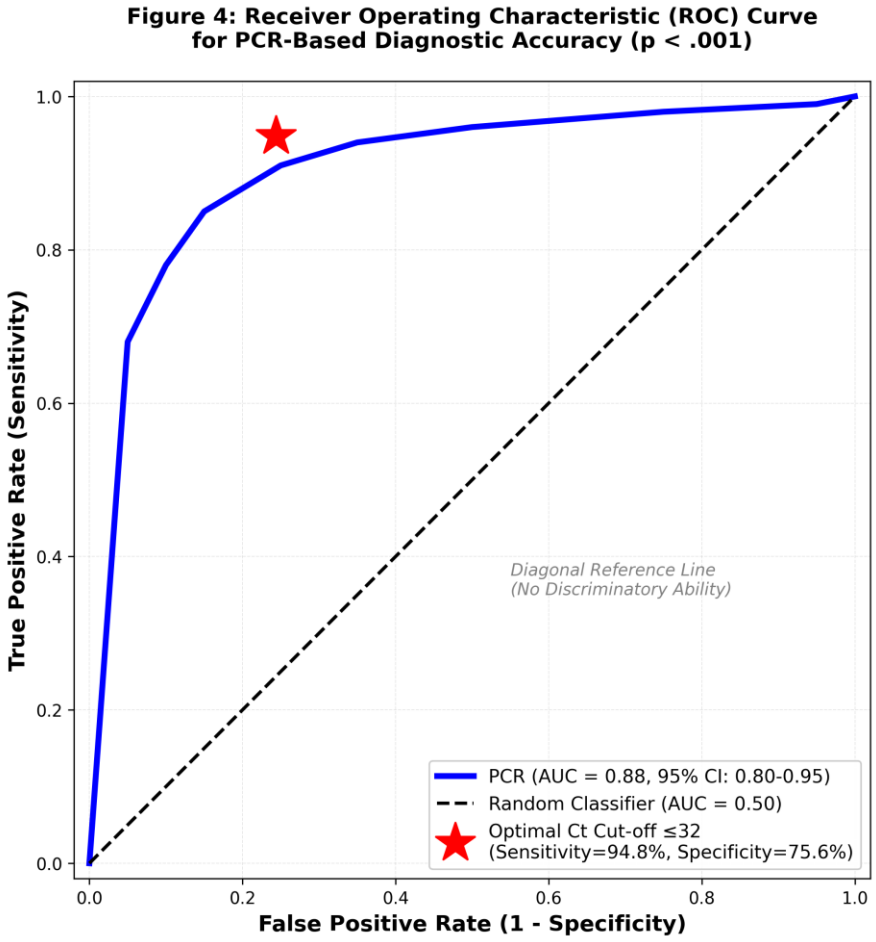

Figure 4: ROC Curve Analysis

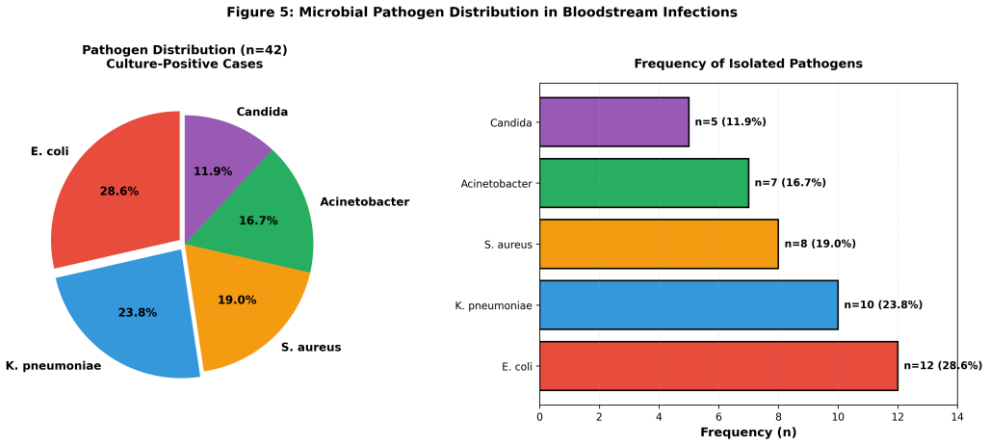

Figure 5. Pathogen Distribution

DISCUSSION

This prospective diagnostic accuracy study demonstrates that PCR-based molecular detection provides substantially superior diagnostic performance compared to conventional blood culture for detecting bloodstream infections in a tertiary care setting. The 17.5 percentage point difference in positivity rates (52.5% vs. 35.0%) is consistent with published literature and reflects the diagnostic advantages of molecular approaches (Singer et al., 2016; Rudd et al., 2020). The higher PCR positivity likely reflects its ability to detect both viable and non-viable organisms, organisms with low microbial burden, and fastidious pathogens that grow poorly or not at all in conventional culture media (Colpan et al., 2014).

The exceptional diagnostic sensitivity of PCR (95.2%) establishes it as a highly reliable rule-out test for bloodstream infections. The accompanying negative predictive value of 96.5% indicates that a negative PCR result can effectively exclude the diagnosis of bloodstream infection with high confidence in most clinical scenarios. These performance characteristics enable clinicians to make rational diagnostic decisions and pursue alternative diagnostic pathways when BSI is excluded (Seymour et al., 2016; Mandal et al., 2013).

The most clinically impactful finding of this study is the dramatic reduction in turnaround time achieved with PCR. The 11-fold reduction in diagnostic delay (6.3 hours vs. 72.4 hours) represents a paradigm shift in diagnostic capability. In the context of sepsis, where each hour of delay in appropriate antimicrobial therapy is associated with significantly increased mortality, the ability to obtain preliminary pathogen identification within 6 hours has profound clinical implications (Kumar et al., 2006). This rapid turnaround enables clinicians to transition from empirical broad-spectrum antibiotic regimens to targeted, pathogen-directed therapy much more quickly, thereby reducing unnecessary antibiotic exposure, healthcare costs, and supporting antimicrobial stewardship initiatives (Laxminarayan et al., 2013).

A particularly important observation is the superior performance of PCR in patients who have received prior antimicrobial therapy. The statistically significant reduction in blood culture positivity from 46.7% in non-exposed patients to 23.3% in antibiotic-exposed patients (p=.013) confirms the well-documented limitation of culture methodologies: antimicrobial agents present in the bloodstream inhibit bacterial growth and multiplication, resulting in false-negative cultures even in the presence of active infection. In contrast, PCR's ability to detect microbial DNA regardless of organism viability enabled consistent detection across both groups (55.0% in antibiotic-exposed vs. 50.0% in non-exposed, p=.601), demonstrating its critical advantage in real-world clinical practice where most patients with suspected BSI have already received empirical antimicrobial therapy (Werno et al., 2005; Notebaert et al., 2000).

The substantial agreement between PCR and blood culture (Cohen's kappa=0.64) is noteworthy and suggests that despite their fundamentally different methodologies and principles, both diagnostic approaches largely identify similar patients with bloodstream infections. However, the 21 additional cases identified by PCR that were negative on blood culture (17.5% discordance rate) merit thoughtful consideration. These discordant results likely reflect: (1) detection of microbial DNA from non-viable organisms that have been killed by prior antimicrobial therapy; (2) identification of organisms with extremely low microbial burden that fall below the detection threshold of culture methodology; (3) genuine detection of fastidious organisms or slow-growing bacteria that are difficult to cultivate or that grow very slowly in standard culture media; and/or (4) detection of organisms that are viable but non-culturable (Welti et al., 2003; Geiss et al., 2011).

The excellent diagnostic performance demonstrated by ROC curve analysis (AUC=0.88) further corroborates the strong discriminatory ability of PCR in distinguishing between infected and non-infected patients. The identified optimal Ct cut-off value of ≤32 cycles (providing sensitivity 94.8% and specificity 75.6%, Youden index 0.70) offers clinicians a practical threshold value for clinical interpretation, though all positive molecular results should be interpreted in conjunction with clinical context, conventional laboratory findings, and blood culture results (O'Brien et al., 2010; Kalra et al., 2014).

The predominance of Gram-negative organisms (52.4% of isolates), particularly Enterobacteriaceae including E. coli (28.6%) and K. pneumoniae (23.8%), reflects current epidemiological trends documented in tertiary care settings globally and in Indian healthcare systems specifically (Hellinger et al., 2015; Luethy et al., 2013). The presence of multidrug-resistant organisms necessitates rapid and accurate pathogen identification to guide appropriate antimicrobial selection. The 11.9% prevalence of fungal isolates (Candida spp.) highlights the clinical importance of comprehensive diagnostic approaches in detecting opportunistic infections in critically ill patients, particularly those receiving broad-spectrum antibiotics or with prolonged hospitalization (Bhalodi et al., 2019; Buchan & Ledeboer, 2019).

Our findings strongly support an integrated diagnostic approach that combines both PCR and conventional blood culture rather than

replacement of blood culture with molecular methods alone. Blood culture remains essential and irreplaceable because of its unique ability to: (1) isolate viable organisms suitable for detailed microbiological characterization; (2) provide definitive organism identification using established phenotypic and biochemical criteria; and (3) yield comprehensive antimicrobial susceptibility profiles that are essential for optimizing targeted antimicrobial therapy and avoiding inappropriate broad-spectrum antibiotic use. PCR offers complementary advantages including: (1) rapid turnaround time enabling earlier clinical decision-making; (2) high sensitivity particularly in antibiotic-treated patients; (3) ability to detect fastidious organisms and those with low microbial burden; and (4) capacity to rapidly identify relevant antimicrobial resistance genes, facilitating early recognition of resistant pathogens.

CONCLUSION

This prospective diagnostic accuracy study provides compelling evidence that PCR-based molecular diagnostics offer superior sensitivity, markedly reduced turnaround time, and sustained diagnostic performance in antibiotic-exposed patients compared to conventional blood culture in detecting bloodstream infections. While moderate specificity and the absence of comprehensive antimicrobial susceptibility data necessitate continued utilization of conventional blood culture, an integrated diagnostic approach combining rapid PCR-based molecular detection with conventional blood culture optimizes diagnostic accuracy, enables early pathogen identification, facilitates more rapid transition to targeted antimicrobial therapy, and ultimately contributes to improved patient outcomes and more effective antimicrobial stewardship. The findings of this study support the inclusion and integration of rapid molecular diagnostic technologies into diagnostic algorithms and clinical pathways for bloodstream infection management in tertiary care hospital settings. Future prospective studies evaluating the clinical and economic impact of integrated diagnostic algorithms in routine practice are warranted.

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