

Original Research Article

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Comparative Study for Rapid Identification of Pathogenic Organisms in Blood Culture by Brain Heart Infusion Broth and Colorcult at a Tertiary Care Hospital, Hyderabad, India

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ABSTRACT

Sepsis remains a critical global health challenge, particularly in resource-limited regions where high-cost automated diagnostic systems are unavailable. To compare the diagnostic performance of ColorCult blood culture bottles with conventional Brain Heart Infusion (BHI) broth for rapid identification of bloodstream pathogens. A prospective comparative study was conducted between January 2024 and December 2024 at a tertiary care hospital in Hyderabad. One hundred blood samples from suspected sepsis patients were parallel-inoculated into both systems and monitored for up to seven days; positive samples were identified and antibiotic susceptibility testing (AST) was performed. From a total of 100 cultures, 33 yielded positive results, with Gram-negative pathogens, specifically *Klebsiella* species (27.3%), being the most prevalent. The primary finding was a significant reduction in the Time to Positivity (TTP); ColorCult bottles detected microbial growth approximately 10–15 hours faster than the BHI method across all patient age groups. While multidrug resistance was common among Gram-negative isolates, Gram-positive organisms maintained high sensitivity to vancomycin and linezolid. The study results demonstrate that ColorCult bottles provide a rapid, reliable, and affordable diagnostic tool for underserved healthcare settings. By substantially shortening the turnaround time for pathogen identification, this method facilitates earlier targeted antimicrobial therapy, which is essential for reducing sepsis-related mortality and improving antimicrobial stewardship.

Keywords

Sepsis, ColorCult, BHI broth, Time to Positivity, Bloodstream Infection, Resource-limited settings.

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Introduction

Sepsis is a life-threatening syndrome resulting from a dysregulated host response to infection, and it remains a major global health burden. According to the Global

Burden of Disease study, sepsis contributes to millions of deaths annually, with the highest incidence and mortality observed in low- and middle-income countries. Early and accurate diagnosis of bloodstream infections is essential for reducing sepsis-related mortality, as emphasized by

the World Health Organization (WHO) and the Surviving Sepsis Campaign, both of which recommend timely blood culture sampling as part of the initial diagnostic bundle.

Automated blood culture systems such as BACTEC and BacT/ALERT have revolutionized sepsis diagnostics in high-income regions by providing rapid and reliable pathogen detection. However, their high cost, maintenance requirements, and dependence on uninterrupted power supply render them impractical in many low-resource laboratories. Conventional methods using Brain Heart Infusion (BHI) broth remain widely employed, but they are hampered by slow turnaround times, labor-intensive sub culturing, and increased risk of contamination.

In this context, ColorCult blood culture bottles represent a promising alternative. These bottles are affordable, user-friendly, and incorporate colorimetric indicators that allow rapid visual detection of microbial growth without specialized equipment. By bridging the gap between conventional manual techniques and advanced automated systems, ColorCult bottles offer a feasible solution for improving sepsis diagnostics in underserved healthcare settings. Their adoption could enable earlier intervention, enhance antimicrobial stewardship, and ultimately reduce the high burden of sepsis-related mortality in resource-constrained regions.

The present study aimed to compare the diagnostic performance of ColorCult blood culture bottles with conventional Brain Heart Infusion (BHI) broth for the rapid identification of pathogenic microorganisms in blood cultures. The study further sought to evaluate the time required for the detection of microbial growth in both culture systems, with particular emphasis on the early diagnosis of bloodstream infections among suspected sepsis cases. In addition, the study assessed the ease of interpretation and the risk of contamination associated with the two methods, particularly in low-resource laboratory settings. Furthermore, the investigation aimed to determine the clinical utility of ColorCult bottles as a cost-effective alternative to automated blood culture systems for improving sepsis diagnosis in resource-constrained environments. The study also evaluated the antimicrobial susceptibility patterns of Gram-positive and Gram-negative bacterial isolates to provide valuable information for appropriate antimicrobial therapy and infection management.

Materials and Methods

Study Design & Setting

This prospective comparative study was conducted in a microbiology laboratory at a tertiary care Osmania General Hospital in Telangana state. The study duration spanned from January 2025 to December 2025.

Study Population

Inclusion Criteria: Patients with clinical suspicion of sepsis or bloodstream infection presenting with fever, chills, hypotension, or other sepsis indicators.

Exclusion Criteria: Patients already on prolonged antimicrobial therapy (>48 hours) or those with inadequate blood sample volume.

Sample Collection & Inoculation

Blood samples were collected under strict aseptic precautions from patients prior to the initiation of antibiotic therapy.

A standard volume of 5–10 mL of venous blood was obtained per culture bottle. Each patient sample was parallel-inoculated into two separate systems: a ColorCult blood culture bottle and a conventional BHI broth bottle.

Culture Conditions & Monitoring

Both culture systems were incubated at 37°C and observed daily for up to 7 days.

ColorCult system: Monitored daily for colorimetric changes indicating active microbial growth.

Conventional BHI broth system: Monitored daily for visible turbidity and colony growth, followed by manual subculture onto solid media.

Identification and Antimicrobial Susceptibility Testing (AST)

Positive cultures from either system were subjected to Gram staining and standardized biochemical tests for definitive pathogen identification. The time to positivity

(TTP) and contamination rates were recorded for each system.

Antibiotic Susceptibility Testing (AST) was performed for all positive clinical isolates in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.

Statistical Analysis

The primary outcome measured was the time to detection of microbial growth. Secondary outcomes included diagnostic yield, contamination rates, and ease of interpretation. Statistical analysis was executed using descriptive statistics and appropriate comparative tests (e.g., Chi-square for categorical variables, t-test for mean detection times).

Results and Discussion

A total of 100 blood cultures were performed, of which 33 yielded confirmed positive results. Demographic analysis of the positive cases revealed that 17 were males (52%) and 16 were females (48%). In terms of age distribution, the majority fell within the 20–40 years cohort (39.4%), followed by the 40–60 years cohort (27.3%). The majority of the positive isolates originated from medical intensive care units, specifically the AMC ward (12 cases, 36.4%) and the IMC ward (9 cases, 27.3%), with the remainder distributed across the SICU, Hemodialysis unit, Surgical gastroenterology unit, Postoperative wards, and RICU.

Time to Positivity (TTP) Comparison

The recorded TTP demonstrated that ColorCult provided significantly earlier and more consistent detection of bloodstream infections compared to BHI across all patient age groups, slashing turnaround time by approximately 10–15 hours.

Pathogen Distribution

Gram-negative organisms predominated in this study, accounting for more than half of the total isolates. *Klebsiella* species were the most frequently isolated pathogen (9 cases, 27.3%), followed by Coagulase-negative *Staphylococcus* (CONS) (7 cases, 21.2%).

Clinical Indices and Outcomes

Multidrug-Resistant Organisms (MDROs) accounted for 27.3% of the cases. Hospital-Acquired Infections (HAIs) constituted 51.52% of the positive cases. Notably, clinical adherence to AST-guided therapy was observed in 69.7% of the patient cohort.

This distribution highlights the dominance of *Klebsiella* and CONS in bloodstream infections among critically ill patients. The lower isolation rate of *S. aureus* (1 case, 3%) suggests that Gram-positive bacteremia was less frequent in this cohort compared to Gram-negative infections.

Antimicrobial Susceptibility Testing

Gram-positive organisms exhibited high overall susceptibility, with *Staphylococcus aureus* showing no resistance to the panel tested. Conversely, extensive multidrug resistance was flagged among Gram-negative isolates, particularly within *Klebsiella* and *Acinetobacter* species.

Blood culture isolates demonstrated highly diverse susceptibility patterns. *Pseudomonas* species retained sensitivity to carbapenems (meropenem), ciprofloxacin, aminoglycosides, and piperacillin-tazobactam, while demonstrating clear resistance to cephalosporins like cefotaxime and ceftriaxone, alongside imipenem and ampicillin. *Escherichia coli* remained sensitive to carbapenems, aminoglycosides, and piperacillin-tazobactam, but displayed resistance to older cephalosporins, ampicillin, co-trimoxazole, and amoxicillin-clavulanate. *Klebsiella* species exhibited pronounced multidrug resistance, particularly against standard cephalosporins and penicillin's, though carbapenems, aminoglycosides, and piperacillin-tazobactam remained effective options.

Acinetobacter species showed resistance to multiple beta-lactams and carbapenems, yet remained sensitive to fluoroquinolones, aminoglycosides, and piperacillin-tazobactam. Gram-positive isolates, including *Enterococcus* species and CONS, showed robust susceptibility to glycopeptides and oxazolidinones (vancomycin, linezolid, teicoplanin) alongside doxycycline and fluoroquinolones, despite notable resistance against macrolides, clindamycin, and ampicillin. *Staphylococcus aureus* remained completely

sensitive to all primary anti-Gram-positive agents with zero resistance recorded in this specific dataset.

Bloodstream infections present a critical challenge in clinical practice, particularly given that sepsis accounts for millions of deaths annually worldwide. Early identification of pathogens and their corresponding antimicrobial susceptibility profiles is essential to steer appropriate targeted therapy and lower mortality. In resource-limited settings, the absence of high-cost automated blood culture systems (such as BACTEC or BacT/ALERT) routinely forces reliance on conventional manual methods like BHI broth. While BHI is inexpensive and widely available, it is significantly hampered by delayed detection, labor-intensive manual sub culturing loops, and higher contamination risk.

Our comparative evaluation highlights that ColorCult blood culture bottles offer major diagnostic advantages over conventional BHI broth. The incorporation of colorimetric indicators permits rapid visual detection of microbial growth at an early stage, effectively compressing turnaround time and enabling earlier initiation of targeted antimicrobial therapy. This timeline acceleration is particularly crucial for aggressive pathogens such as *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Acinetobacter* spp., which frequently harbor multidrug resistance phenotypes and require immediate, appropriate clinical intervention.

The high sensitivity maintained by *Enterococcus* spp. and *Staphylococcus aureus* to vancomycin and linezolid underscores the value of rapid culture confirmation; getting fast visual verification prevents inappropriate empirical over-prescription and preserves these critical last-line agents. These findings strongly align with WHO recommendations highlighting timely blood culture sampling as a crucial element of the sepsis care bundle.

Moreover, Surviving Sepsis Campaign guidelines emphasize that cumulative delays in initiating effective antimicrobial therapy directly increase patient mortality. By successfully bridging the operational gap between slow manual methodologies and costly advanced automated systems, ColorCult bottles offer a feasible, cost-effective diagnostic upgrade for resource-

constrained laboratories.

Broad implementation of this approach could significantly strengthen local antimicrobial stewardship, optimize AMR prevention strategies, and ultimately lower sepsis-related mortality rates.

In conclusion, Sepsis remains a major global health challenge, particularly in low- and middle-income countries where diagnostic infrastructure is limited and delays in pathogen identification contribute significantly to high mortality.

Conventional BHI broth, though widely used, is hindered by slow detection and high contamination risk. In contrast, ColorCult blood culture bottles provide a simple, affordable, and effective alternative by enabling rapid visual detection of microbial growth without the need for automated systems.

Our comparative evaluation demonstrates that ColorCult bottles can shorten turnaround times, reduce laboratory workload, and improve diagnostic accuracy for bloodstream infections.

Adoption of this method in resource-constrained laboratories could enhance antimicrobial stewardship, facilitate timely initiation of targeted therapy, and ultimately reduce sepsis-related morbidity and mortality.

Limitations of the Study

This study has a few limitations that should be acknowledged:

The sample size was relatively small (n = 100), due to the restricted availability of ColorCult bottles at the time of the study.

The evaluation was conducted within a single laboratory inside a single resource-limited institutional environment.

The study did not include a direct parallel comparison with automated systems such as BACTEC or BacT/ALERT, which remain the gold standard in high-resource settings.

Table.1 Comparison of mean Time to Positivity (TTP) between ColorCult and BHI bottles across age groups

Age Group (years)	n DOCX	ColorCult, mean ± SD (h) DOCX	BHI, mean ± SD (h) DOCX	Mean Difference (h) DOCX
0–20	3	13.3 ± 3.5	24.0 ± 12.5	10.7
20–40	13	15.1 ± 3.8	26.7 ± 15.2	11.6
40–60	9	16.2 ± 4.1	29.5 ± 17.8	13.3
60–80	8	14.8 ± 4.0	28.2 ± 16.1	13.4

Note: Values are expressed as mean ± SD. TTP = time to positivity; h = hours; SD = standard deviation.

Table.2 Pathogenic organisms isolated in positive blood culture cases

Organism Isolated	Total Number	Percentage
<i>Klebsiella</i> species	9	27.3%
Coagulase-negative <i>Staphylococcus</i> (CONS)	7	21.2%
<i>Escherichia coli</i>	5	15.1%
<i>Pseudomonas</i> species	4	12.1%
<i>Enterococcus</i> species	4	12.1%
<i>Acinetobacter</i> species	3	9.1%
<i>Staphylococcus aureus</i>	1	3.0%

Table.3 MDRO status among positive cases

Category	Frequency (n)	Percentage (%)
MDRO Positive	9	27.3
MDRO Negative	24	72.7

Table.4 Hospital-Acquired Infection (HAI) rate among positive cases

Category	Frequency (n)	Percentage (%)
HAI Yes	17	51.52
HAI No	16	48.48

Table.5 Adherence to AST-guided therapy among positive cases

Category	Frequency (n)	Percentage (%)
Yes	23	69.7
No	10	30.3

Table.6 Clinical outcome among positive sepsis cases

Outcome	Frequency (n)	Percentage (%)
Discharge	24	72.7
Death	9	27.2

Table.6 Antimicrobial Susceptibility Testing

Organism	Sensitive Antibiotics	Resistant Antibiotics
<i>Pseudomonas</i> species (incl. <i>P. aeruginosa</i>)	CIP, MRP, CPM, AT, GEN, PIT, AK, COT, LE, CAZ, AMC	CTX, IPM, AMP, CTR
<i>Escherichia coli</i>	AK, GEN, CPM, CTX, MRP, ETP, OF, CFS, PIT	CFM, AMP, CAC, CX, COT, AMC, CTR
<i>Klebsiella</i> species	AK, GEN, COT, CPM, MRP, IPM, ETP, PIT, CX, AMC, CAZ, CTR	CTX, AMP, CFM, CAC, CIP
<i>Acinetobacter</i> species	PIT, OF, MRP, CPM, CIP, COT, AK	GEN, CAC, AMC, CAZ, CTR, IPM, CFM, CX, CTX, AMP
<i>Enterococcus</i> species	VA, LZ, TE, DO, OF, TEI, HLG	AMP, E, CIP
Coagulase-negative <i>Staphylococcus</i> (CONS)	VA, LZ, DO/DOX, TE, TEI, GEN, CX, COT, FR, AZM, CD, CIP, OF	AZM, CIP, E, CD, COT, CX, GEN
<i>Staphylococcus aureus</i>	VA, LZ, DO, TEI, AZM	NIL

Antibiotic Key: AK: Amikacin; AMC: Amoxicillin-Clavulanate; AMP: Ampicillin; AT: Aztreonam; AZM: Azithromycin; CAC: Ceftazidime/Clavulanate; CAZ: Ceftazidime; CD: Clindamycin; CFM: Cefixime; CFS: Cefoperazone-Sulbactam; CIP: Ciprofloxacin; COT: Co-trimoxazole; CPM: Cefepime; CTR: Ceftriaxone; CTX: Cefotaxime; CX: Cloxacillin/Cefoxitin; DO/DOX: Doxycycline; E: Erythromycin; ETP: Ertapenem; FR: Furazolidone; GEN: Gentamicin; HLG: High-Level Gentamicin; IPM: Imipenem; LE: Levofloxacin; LZ: Linezolid; MRP: Meropenem; OF: Ofloxacin; PIT: Piperacillin-Tazobactam; TE: Tetracycline; TEI: Teicoplanin; VA: Vancomycin.

Future Directions

Future studies should aim to address the limitations identified in this work:

Larger, multicenter trials across diverse geographic and clinical settings would improve the generalizability of findings and provide stronger evidence for the utility of ColorCult blood culture bottles in sepsis diagnostics.

Incorporating advanced molecular techniques such as PCR or MALDI-TOF MS alongside conventional biochemical methods could enhance pathogen identification accuracy and allow detection of fastidious organisms that may be missed by culture alone.

Comparative evaluations including automated systems such as BACTEC or BacT/ALERT would provide a more comprehensive benchmark, highlighting the relative strengths and weaknesses of ColorCult in different laboratory contexts.

Expanding antimicrobial susceptibility testing to include newer agents and reserve antibiotics would offer deeper insights into resistance trends and guide stewardship strategies. Finally, cost-effectiveness analyses and

implementation studies in real-world low-resource laboratories are needed to determine the practical feasibility, sustainability, and impact of ColorCult adoption on sepsis-related outcomes.

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Author Contributions

Dr. G. V. Padmaja: Conceptualization, study design, methodology development, final draft writing, project administration, and supervision. Dr. Geetha Kaipa: Validation, formal data analysis, quality assurance, visualization, and reviewing & editing the manuscript draft. Dr. G. Preethi: Resources, laboratory investigation, data curation, software analysis. Dr. Ajaz Ahmed: Sample collection, day-to-day experimental work, processing of blood cultures, biochemical testing, antimicrobial susceptibility testing (AST) and original draft preparation.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval: Approved by the institutional ethics committee.

Conflict of Interest: The authors declare no conflict of interest.

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