

Diagnostic Performance of Conventional and Modified Loop-Mediated Isothermal Amplification for Detection of *Burkholderia cepacia* in Bloodstream Infections: A Prospective Cross-Sectional Study

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ABSTRACT

Background:

Burkholderia cepacia complex (BCC) is an opportunistic, multidrug-resistant Gram-negative pathogen associated with healthcare-associated bloodstream infections. Rapid identification is clinically important, while conventional culture requires prolonged processing.

Objective:

To compare the diagnostic performance of conventional and modified loop-mediated isothermal amplification (LAMP) assays for detection of *B. cepacia* using conventional blood culture as the reference standard and to describe the antimicrobial susceptibility profile of culture-confirmed isolates.

Methods:

In this prospective cross-sectional diagnostic accuracy study, 310 blood specimens from patients with suspected bloodstream infection were evaluated by conventional culture, conventional LAMP, and modified LAMP. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated. Antimicrobial susceptibility testing was performed for culture-confirmed isolates.

Results:

Culture identified 67/310 (21.6%) *B. cepacia*-positive specimens. Conventional LAMP showed 76.12% sensitivity, 100% specificity, 100% PPV, 93.82% NPV, and 94.84% accuracy. Modified LAMP showed 73.13% sensitivity, 100% specificity, 100% PPV, 93.10% NPV, and 94.19% accuracy. Meropenem showed the highest in-vitro activity, whereas resistance was comparatively high to cefoperazone and ciprofloxacin.

Conclusion:

Both LAMP assays demonstrated excellent specificity and high overall accuracy. Conventional LAMP showed marginally better sensitivity and accuracy than the modified assay. LAMP may serve as a rapid adjunct to culture, while culture remains essential for confirmation and antimicrobial susceptibility testing.

Keywords: *Burkholderia cepacia* complex; bloodstream infection; loop-mediated isothermal amplification; LAMP; diagnostic accuracy; antimicrobial susceptibility.

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Introduction

Bloodstream infections (BSIs) remain one of the leading causes of morbidity and mortality worldwide and constitute a major challenge for healthcare systems because of their association with prolonged hospitalization, increased healthcare costs, and poor clinical outcomes. Rapid progression from bacteraemia to sepsis and septic shock necessitates timely identification of the causative pathogen and prompt initiation of appropriate antimicrobial therapy. Delayed diagnosis or inappropriate empirical treatment has been consistently associated with increased mortality, particularly among critically ill and immunocompromised patients. Consequently, the development of rapid and reliable diagnostic methods has become a priority in clinical microbiology and infectious disease management [1–4].

Among the non-fermentative Gram-negative bacilli, the *Burkholderia cepacia* complex (BCC) has emerged as an important opportunistic pathogen responsible for a wide range of healthcare-associated infections. Initially identified by Walter H. Burkholder in 1950 as the causative agent of onion bulb soft rot, the organism was subsequently reclassified from the genus *Pseudomonas* to *Burkholderia* following advances in molecular taxonomy. The BCC currently comprises more than twenty genetically distinct but phenotypically similar species, many of which possess significant pathogenic potential in humans. Although several members of the complex are harmless environmental organisms, others are increasingly recognized as important causes of bloodstream infections, respiratory tract infections, urinary tract infections, surgical site infections, and catheter-related infections, particularly among hospitalized patients with underlying medical conditions [5–8].

The clinical importance of BCC is closely related to its remarkable ecological adaptability and intrinsic antimicrobial resistance. Members of this complex are widely distributed in soil, water, and plant rhizospheres and possess the ability to survive under nutrient-limited conditions and in environments containing disinfectants, antiseptics, and pharmaceutical solutions. This exceptional environmental persistence is facilitated by biofilm formation, multidrug efflux pumps, reduced outer membrane permeability, and production of hydrolytic enzymes, enabling the organism to colonize medical devices and persist in hospital environments. Numerous healthcare-associated outbreaks have been linked to contaminated intravenous fluids, nebulizer solutions, dialysis fluids, disinfectants, and inadequately sterilized medical equipment, emphasizing the importance of strict infection prevention and environmental surveillance [9–14]. Treatment of BCC infections is particularly challenging because of the organism's intrinsic and acquired resistance to multiple antimicrobial agents.

Resistance to aminoglycosides, polymyxins, and several β -lactam antibiotics substantially limits therapeutic options, while emerging resistance to ceftazidime, meropenem, minocycline, and trimethoprim-sulfamethoxazole has been reported in different geographical regions. These resistance mechanisms are mediated through a combination of β -lactamase production, active efflux systems, altered membrane permeability, and biofilm-associated tolerance, all of which contribute to persistent infections and therapeutic failure. Therefore, rapid laboratory identification combined with antimicrobial susceptibility testing is essential for optimizing patient management and supporting antimicrobial stewardship programmes [15–19].

Despite increasing awareness of BCC as an important nosocomial pathogen, laboratory diagnosis remains challenging. Conventional culture methods continue to serve as the reference standard because they permit recovery of viable organisms for definitive identification and antimicrobial susceptibility testing. However, culture-based diagnosis generally requires 48–72 hours or longer and may delay clinical decision-making in critically ill patients. Furthermore, phenotypic similarities between BCC and other non-fermentative Gram-negative bacilli may result in misidentification when only biochemical or automated identification systems are used. Such diagnostic limitations highlight the need for rapid molecular methods capable of accurately identifying BCC directly from clinical specimens [20–28].

Recent advances in molecular diagnostics have substantially improved the detection of clinically important bacterial pathogens. Techniques such as polymerase chain reaction (PCR), quantitative PCR, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), whole-genome sequencing (WGS), CRISPR-based diagnostics, biosensor technologies, and microfluidic platforms have demonstrated high analytical performance. Nevertheless, many of these methods require sophisticated laboratory infrastructure, expensive instrumentation, and trained personnel, restricting their widespread application in routine diagnostic laboratories, particularly in resource-limited healthcare settings [25,27–32].

Loop-Mediated Isothermal Amplification (LAMP) has emerged as a promising alternative molecular diagnostic technique because it amplifies nucleic acids under isothermal conditions without requiring thermal cycling. The assay employs multiple primers targeting distinct regions of the bacterial genome, providing high analytical specificity, rapid amplification, and visual detection of amplification products within approximately one hour. In comparison with conventional PCR, LAMP is technically simpler, less expensive, and more suitable for point-of-care and decentralized laboratory settings. Recent investigations have demonstrated the

utility of LAMP for detecting numerous bacterial pathogens; however, evidence regarding its clinical performance for the diagnosis of *Burkholderia cepacia* bloodstream infections remains limited, particularly in comparison with conventional LAMP protocols and culture-based diagnosis [33–43].

A review of the available literature indicates several important knowledge gaps. Most published investigations concerning BCC have focused on cystic fibrosis-associated pulmonary disease or outbreak investigations, whereas relatively few studies have evaluated rapid molecular diagnosis of bloodstream infections in non-cystic fibrosis populations. Furthermore, comparative evaluations of modified LAMP assays against conventional LAMP using culture as the reference standard remain scarce, particularly in tertiary-care hospitals within low- and middle-income countries. Addressing these gaps is essential to establish clinically applicable, rapid, and cost-effective diagnostic strategies for BCC infections [1–4,21,44].

Therefore, the present prospective cross-sectional study was undertaken to compare the diagnostic performance of a modified Loop-Mediated Isothermal Amplification assay with conventional LAMP for the detection of *Burkholderia cepacia* in blood culture specimens using conventional culture as the reference standard. The study further aimed to evaluate the diagnostic accuracy of both molecular assays and assess the antimicrobial susceptibility profile of culture-confirmed isolates. The findings are expected to contribute to the growing evidence supporting rapid molecular diagnostics and facilitate earlier diagnosis, timely targeted antimicrobial therapy, and improved infection control practices in patients with suspected bloodstream infections.

Materials and Methods

Study Design and Setting

A prospective cross-sectional diagnostic accuracy study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research (DMIHER), Wardha, Maharashtra, India. The study was performed at Acharya Vinoba Bhave Rural Hospital (AVBRH), a 1,280-bed tertiary-care teaching hospital serving the population of central India. The study was designed to compare the diagnostic performance of conventional Loop-Mediated Isothermal Amplification (LAMP) and a modified LAMP assay for the detection of *Burkholderia cepacia* complex (BCC), using conventional blood culture as the reference standard. The study was planned and reported according to accepted principles for diagnostic accuracy studies [45,46].

Study Population and Sample Size

Patients with clinical suspicion of bloodstream infection or sepsis who underwent blood culture as part of routine clinical management were eligible. Clinical suspicion was based on features of systemic infection, including fever, chills, hypotension, tachycardia, leukocytosis or leukopenia, elevated inflammatory markers, or other manifestations

considered compatible with sepsis by the treating physician. Patients admitted to intensive care units and medical, surgical, respiratory medicine, obstetrics and gynaecology, paediatric, orthopaedic, and other inpatient departments were considered for enrolment. Patients were included when an adequate blood specimen was available for both conventional microbiological and molecular testing. Specimens that were inadequate in volume, improperly collected or contaminated, duplicate samples from the same infectious episode, or samples with insufficient clinical information were excluded. A total of 310 blood specimens were included. Each specimen was evaluated by conventional culture, conventional LAMP, and modified LAMP, and the two molecular assays were compared with culture for calculation of diagnostic performance.

Blood Sample Collection and Conventional Culture

Approximately 5–10 mL of venous blood was collected by trained healthcare personnel under strict aseptic precautions after appropriate skin antisepsis. Blood was inoculated into automated blood-culture bottles and transported promptly to the microbiology laboratory. Culture bottles were incubated using the automated blood-culture system employed by the institutional laboratory until flagged positive or until completion of the routine incubation period. Positive bottles were subcultured onto blood agar and MacConkey agar and incubated aerobically. Growth suggestive of non-fermenting Gram-negative bacilli was evaluated by Gram staining, colony morphology, oxidase and catalase reactions, and other conventional biochemical tests used in the laboratory. Culture-confirmed *B. cepacia* isolates were considered positive and conventional culture served as the reference standard for subsequent comparison of the molecular assays [20–24].

DNA Preparation

DNA was prepared from the clinical material or recovered isolates according to the laboratory extraction protocol used during the study. Extracted DNA was used as the template for both conventional and modified LAMP. Template preparation, reagent handling, amplification, and post-amplification procedures were carried out in designated work areas to reduce the risk of cross-contamination. Positive-control DNA and a no-template negative control were included in each analytical run.

LAMP Primer Design

A BCC-specific LAMP primer set targeting the *ribB5* region was selected from the published assay of Daddy Gao et al. [39]. The primer system consisted of two outer primers (F3 and B3), two inner primers (FIP and BIP), and two loop primers (LF and LB). Use of loop primers can accelerate LAMP amplification by providing additional sites for strand-displacement synthesis [34]. The *RibB5* primer set was selected because the published assay demonstrated high specificity for BCC and used a conserved *ribB* target suitable for isothermal amplification [39]. The primer sequences used in the present study are shown in Table 1.

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Primer name	Primer sequence (5'–3')	Size (bp)
RibB5 F3	GGCCGGATGGTGATCCT	17
RibB5 B3	GTCATCAGCGGCAGGTG	17
RibB5 FIP	TCCGGTGTGACGAATTCGGCCGAA GAAGACCGCGAAAACG	40
RibB5 BIP	AACTTCATGGCCAAGTACGGCCAA GCTGCTTGACGCGTT	39
RibB5 LF	GATCACGAGGTCGCCCT	17
RibB5 LB	CGGCCTGGTTTGTCTGA	17

Abbreviations: F3, forward outer primer; B3, backward outer primer; FIP, forward inner primer; BIP, backward inner primer; LF, loop forward primer; LB, loop backward primer.

Optimization of LAMP Conditions

LAMP conditions were optimized using positive-control DNA at 100 ng/μL. The optimization procedure assessed reaction temperature, magnesium concentration, and incubation time. Five reaction temperatures (57°C, 59°C, 61°C, 63°C, and 65°C) were evaluated. Magnesium concentration was assessed over a range of 1–8 mM while the concentrations of the remaining reaction components were maintained constant. After selection of suitable temperature and magnesium conditions, reaction times of 10, 20, 30, 40, 50, and 60 minutes were evaluated. The study protocol used outer primers F3 and B3 at 0.4 μM each, inner primers FIP and BIP at 1.6 μM each, loop primers LF and LB at 0.8 μM each, together with magnesium salt, betaine, dNTPs, strand-displacing DNA polymerase, reaction buffer, and template DNA. These optimization steps were undertaken to identify conditions that produced reproducible amplification while minimizing non-specific reactions. LAMP is inherently suited to such optimization because amplification proceeds at a single temperature through strand-displacing polymerase activity [33–41].

Conventional LAMP Assay

Conventional LAMP was performed using the RibB5 six-primer system under isothermal conditions. The reaction mixture used the primer concentrations described above together with magnesium salt, betaine, dNTPs, strand-displacing DNA polymerase, reaction buffer, and template DNA. The published RibB5 method used as the methodological reference employed a 10-μL WarmStart colorimetric LAMP reaction at 65°C for 25 minutes [39]. In the colorimetric approach, amplification is detected through the pH-sensitive phenol-red system:

successful amplification produces a visible change from red/pink to yellow, while a reaction retaining the original red/pink colour is interpreted as negative. The published assay also demonstrated that the RibB5 primer set could specifically detect BCC and that colorimetric results could be confirmed by amplification-product analysis [39]. In the present study, appropriate positive and no-template negative controls were included in every run. A test result was accepted only when the positive control amplified appropriately and the negative control remained negative. General principles of LAMP amplification and visual endpoint detection were consistent with established LAMP methodology [33–36,40,41].

Modified LAMP Assay

The modified LAMP assay used the same BCC-specific RibB5 target while incorporating optimization of reaction and detection conditions to permit fluorescence-based monitoring. Reaction temperature, incubation time, magnesium concentration, and primer conditions were evaluated as described above. EvaGreen, a double-stranded DNA-binding fluorescent dye, was incorporated into the modified reaction for real-time monitoring of amplification. As LAMP products accumulated, binding of EvaGreen to amplified double-stranded DNA generated an increase in fluorescence intensity. A sample showing a characteristic rise in fluorescence during the amplification period was interpreted as positive, provided that the positive control showed the expected amplification and the no-template control remained negative. Samples without a detectable amplification signal were interpreted as negative. This fluorescence-based readout enabled amplification to be followed during the reaction rather than relying solely on visual endpoint colour change. The modified LAMP results were recorded independently and subsequently compared with conventional LAMP and the culture reference standard. Because fluorescence thresholds and time-to-positive criteria are instrument- and protocol-dependent, the final instrument-specific threshold used for routine interpretation should be reported exactly from the laboratory analytical record when the manuscript is submitted.

Antimicrobial Susceptibility Testing

All culture-confirmed *B. cepacia* isolates underwent antimicrobial susceptibility testing according to the routine laboratory protocol. Kirby-Bauer disk diffusion was performed using the interpretive criteria applicable during the study period. The antimicrobial panel included ceftazidime, meropenem, minocycline, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, cefoperazone, and cefoperazone-sulbactam. Zone diameters were interpreted according to the laboratory standard in use, and susceptibility profiles were summarized as frequencies and percentages. Because BCC demonstrates substantial intrinsic and acquired antimicrobial resistance, phenotypic susceptibility testing was retained as an essential component of the study [15–19].

Quality Control and Data Collection

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Standard operating procedures were followed for specimen collection, transportation, culture processing, molecular testing, and antimicrobial susceptibility testing. Positive and negative controls were included in each LAMP run to monitor assay performance and contamination. Culture media were quality checked before use and laboratory equipment was maintained according to institutional quality-assurance procedures. Demographic characteristics, clinical information, culture findings, LAMP results, and antimicrobial susceptibility profiles were recorded using a structured data-collection form. Each participant was assigned a unique study identification number and personal identifiers were excluded from the analytical dataset.

Statistical Analysis

Conventional blood culture was considered the reference standard. Conventional and modified LAMP results were classified as true positive, true negative, false positive, or false negative relative to culture. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy were calculated with 95% confidence intervals where applicable. Continuous variables were summarized using mean $\pm$ standard deviation or median with interquartile range according to distribution, while categorical variables were expressed as frequencies and percentages. Comparative analysis of paired diagnostic results was performed using an appropriate test for paired categorical data, and $P < 0.05$ was considered statistically significant. Diagnostic accuracy reporting followed STARD recommendations [45,46].

Ethical Considerations

Ethical approval was obtained from the Institutional Ethics Committee of Datta Meghe Institute of Higher Education and Research before commencement of the study (DMIHER(DU)/IEC/2025/563). All study procedures involving human participants were conducted in accordance with approved institutional protocols and applicable ethical standards.

Results

A total of 310 blood culture specimens obtained from patients with clinically suspected bloodstream infections were included in this prospective cross-sectional study. All specimens were processed using conventional blood culture (reference standard), conventional Loop-Mediated Isothermal Amplification (LAMP), and modified LAMP to evaluate and compare their diagnostic performance for the detection of *Burkholderia cepacia* complex (BCC). Demographic characteristics, ward distribution, diagnostic accuracy parameters, and antimicrobial susceptibility profiles were analysed.

Gender Distribution

Among the 310 patients included in the study, 159 (51.3%) were males and 151 (48.7%) were females. Blood culture identified 67 positive cases of *Burkholderia cepacia*, corresponding to an overall culture positivity rate of 21.6%, whereas 243 (78.4%) samples were culture negative. The distribution of positive cultures was nearly identical between both

sexes, with 34 (50.7%) positive isolates recovered from male patients and 33 (49.3%) from female patients. This observation suggests that the occurrence of *Burkholderia cepacia* bloodstream infection was comparable in both genders, with no apparent sex-related predominance in the present study. Gender wise distribution are shown in Table 2

Age-wise Distribution

Patients ranged from infancy to more than 75 years of age. The highest proportion of culture-positive cases was observed in the 19–30-year age group, accounting for 19 (28.4%) of all positive isolates. This was followed by the 46–60-year group with 15 (22.4%) positive cultures and the 61–75-year group with 12 (17.9%) cases. Patients aged 31–45 years contributed 11 (16.4%) positive isolates, whereas children aged 0–12 years and adolescents aged 13–18 years accounted for 7.5% and 6.0% of positive cases, respectively. Only one positive isolate (1.4%) was identified among patients older than 75 years. These findings indicate that bloodstream infections due to *Burkholderia cepacia* occurred predominantly among young and middle-aged adults in the present study. Age wise distribution are shown in Table 2

Demographic characteristic	Total samples, n	Culture positive, n	Positive cases, %
Gender			
Male	159	34	50.7
Female	151	33	49.3
Age group (years)			
0–12	23	5	7.5
13–18	11	4	6.0
19–30	83	19	28.4
31–45	71	11	16.4
46–60	72	15	22.4
61–75	46	12	17.9
>75	4	1	1.4
Total	310	67	100.0

Table 2: Gender and age distribution of the study participants and culture positive cases

Ward-wise Distribution of Clinical Samples

Analysis of the distribution of specimens according to hospital wards demonstrated that the majority of blood culture samples originated from the Medicine ward, which contributed 100 (32.25%) specimens. This was followed by the Surgery ward with 80 (25.80%) samples and the Intensive Care Unit (ICU) with 69 (22.25%) samples. Additional specimens were obtained from the Department of Respiratory Medicine (36; 11.61%), Obstetrics and Gynaecology (13; 4.19%), Paediatrics (10; 3.22%), and Orthopaedics (2; 0.64%). The predominance of samples from Medicine, Surgery, and ICU reflects the higher burden of suspected bloodstream infections in these clinical settings.

Ward	Isolation	Percentage (%)
Medicine	100	32.25

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Surgery	80	25.80
ICU	69	22.25
OBGY	13	4.19
Respiratory Medicine	36	11.61
Pediatrics	10	3.22
Orthopedic	02	0.64

Table 3: Ward wise distribution of samples
Comparative Detection of *Burkholderia cepacia*

Conventional blood culture identified 67 positive cases, which served as the reference standard for evaluating both molecular assays. The conventional LAMP assay detected 51 positive cases, whereas the modified LAMP assay detected 49 positive cases. Although both molecular methods successfully identified the majority of culture-confirmed cases, the conventional LAMP assay detected slightly more positive samples than the modified LAMP assay. Nevertheless, both methods demonstrated substantial agreement with the reference standard and provided considerably shorter turnaround times than conventional culture.

Diagnostic Performance of Conventional LAMP

Using conventional culture as the reference standard, the conventional LAMP assay demonstrated a sensitivity of 76.12% (95% CI: 64.14–85.69%) and a specificity of 100.0% (95% CI: 93.02–100.0%). The positive predictive value (PPV) was 100.0%, indicating that every sample identified as positive by conventional LAMP was confirmed by culture. The negative predictive value (NPV) was 93.82% (95% CI: 90.83–95.88%), reflecting a high probability that negative LAMP results represented true negative cases. Overall diagnostic accuracy was 94.84% (95% CI: 91.75–97.02%), demonstrating excellent diagnostic performance of the conventional assay.

Method	Number
Culture positive	67
Regular LAMP positive	51
Modified LAMP positive	49

Table 4: Comparative assessment of Gold Standard method with Regular Lamp Test and Modified Lamp test

Diagnostic Performance of Modified LAMP

The modified LAMP assay demonstrated a sensitivity of 73.13% (95% CI: 60.90–83.24%) and a specificity of 100.0% (95% CI: 93.02–100.0%). Similar to the conventional assay, the positive predictive value was 100.0%, confirming the absence of false-positive results. The negative predictive value was 93.10% (95% CI: 90.09–95.25%), while

the overall diagnostic accuracy was 94.19% (95% CI: 90.98–96.52%). These findings indicate that the modified LAMP assay also demonstrated excellent specificity and overall accuracy but exhibited slightly lower sensitivity than the conventional LAMP assay.

Comparative Diagnostic Accuracy

Direct comparison of the two molecular assays showed only minor differences in diagnostic performance. Conventional LAMP demonstrated a sensitivity of 76.12%, compared with 73.13% for the modified LAMP assay. Both assays achieved 100% specificity, indicating that neither method generated false-positive results in comparison with culture. Similarly, the overall diagnostic accuracies were comparable, measuring 94.84% for conventional LAMP and 94.19% for modified LAMP. Although the conventional assay exhibited marginally better sensitivity and accuracy, both methods demonstrated excellent overall diagnostic performance and high concordance with the reference standard.

Metric	Regular LAMP	Modified LAMP
Sensitivity	76.12%	73.13%
Specificity	100.00%	100.00%
Accuracy	94.84%	94.19%

Table 5: Comparison between the Regular Lamp Test and Modified Lamp Test

Antimicrobial Susceptibility Pattern

Antimicrobial susceptibility testing of the 67 culture-confirmed *Burkholderia cepacia* isolates demonstrated variable susceptibility profiles across the antibiotics evaluated. Meropenem exhibited the highest in vitro activity, with 61 isolates susceptible and only 6 resistant. High susceptibility was also observed for cefoperazone–sulbactam, with 55 susceptible isolates, followed by minocycline, ceftazidime, and co-trimoxazole, each demonstrating susceptibility in 46 isolates. In contrast, susceptibility to levofloxacin was lower, with 33 susceptible and 34 resistant isolates, indicating nearly equal susceptibility and resistance rates.

Among the antimicrobial agents tested, cefoperazone demonstrated the poorest activity, with only 20 susceptible isolates compared with 47 resistant isolates, making it the least effective antibiotic in the present study. Similarly, ciprofloxacin exhibited limited efficacy, with 29 susceptible and 38 resistant isolates. These findings indicate considerable variation in antimicrobial susceptibility among *Burkholderia cepacia* isolates and support the importance of routine susceptibility testing to guide targeted antimicrobial therapy.

Discussion

Burkholderia cepacia complex (BCC) is an increasingly recognized opportunistic pathogen associated with healthcare-associated infections, particularly among hospitalized and critically ill patients. Its clinical importance is enhanced by its ability to survive in hospital environments, form biofilms, and exhibit intrinsic resistance to multiple

antimicrobial agents [5,8–10,15]. Rapid identification is therefore important for early therapeutic intervention and infection-control measures.

In the present study, 67 of 310 blood specimens (21.6%) were culture positive for *B. cepacia*. The distribution of positive cases was almost equal between males and females, suggesting no substantial sex-related predominance. Most specimens were obtained from Medicine, Surgery, and intensive-care settings, where invasive procedures, prolonged hospitalization, indwelling devices, and previous antimicrobial exposure may increase the risk of healthcare-associated infection. Previous investigations have similarly described BCC bacteremia and hospital outbreaks among vulnerable patient populations [1–4,12–14].

The principal finding was the high diagnostic specificity of both LAMP assays. Conventional LAMP demonstrated a sensitivity of 76.12%, specificity of 100%, PPV of 100%, NPV of 93.82%, and overall accuracy of 94.84%. Modified LAMP showed a sensitivity of 73.13%, specificity of 100%, PPV of 100%, NPV of 93.10%, and accuracy of 94.19%. Thus, conventional LAMP showed marginally higher sensitivity and accuracy, whereas both assays demonstrated excellent specificity. The modified assay did not demonstrate superior diagnostic performance under the conditions evaluated in this study.

LAMP offers practical advantages for rapid molecular diagnosis because amplification occurs under isothermal conditions and does not require conventional thermal cycling [33–38,40–43]. Daddy Gaoh et al. demonstrated rapid and specific LAMP-based detection of BCC in non-sterile pharmaceutical products [39]. However, that investigation used pharmaceutical-product matrices, whereas the present study evaluated suspected bloodstream infection. The moderate sensitivity observed here indicates that LAMP should currently be considered an adjunct to, rather than a replacement for, conventional culture.

Antimicrobial susceptibility testing demonstrated considerable variation among BCC isolates. Meropenem showed the highest activity, with 61 of 67 isolates susceptible, followed by cefoperazone-sulbactam. Minocycline, ceftazidime, and cotrimoxazole also retained activity against a substantial proportion of isolates, whereas resistance was comparatively high to cefoperazone and ciprofloxacin. These findings are consistent with the recognized multidrug-resistant nature of BCC and the diversity of resistance phenotypes reported in clinical isolates [15–19]. Therefore, antimicrobial therapy should be guided by isolate-specific susceptibility results whenever possible. Overall, conventional and modified LAMP were highly specific rapid molecular methods for detecting *B. cepacia*, although conventional LAMP demonstrated slightly better sensitivity and overall accuracy. LAMP may complement conventional culture by facilitating earlier presumptive detection, while culture remains

essential for definitive identification and antimicrobial susceptibility testing. Further multicentre studies with standardized sample processing, optimized primer design, and larger patient populations are required.

Conclusion

The present study demonstrated that conventional and modified LAMP are rapid and highly specific molecular methods for detecting *Burkholderia cepacia* complex in suspected bloodstream infections. Both assays achieved 100% specificity and high overall diagnostic accuracy, although conventional LAMP showed slightly higher sensitivity (76.12%) and accuracy (94.84%) than modified LAMP (73.13% and 94.19%, respectively). Antimicrobial susceptibility testing showed that meropenem and cefoperazone-sulbactam had comparatively high activity, whereas greater resistance was observed to cefoperazone and ciprofloxacin. LAMP may therefore serve as a useful rapid adjunct to conventional culture; however, culture remains essential for definitive confirmation and antimicrobial susceptibility testing. Further multicentre studies are required to optimize LAMP sensitivity and validate its routine clinical application for rapid diagnosis of BCC bloodstream infections.

Declarations

Ethics approval and consent to participate: The study was conducted after approval from the Institutional Ethics Committee of Datta Meghe Institute of Higher Education and Research. [DMIHER(DU)/IEC/2025/563]

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