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Original Article

Direct MALDI-TOF MS identification from positive blood culture bottles: a time-saving approach to reduce diagnostic delays

Jyotsna Agarwal[1], Nikhil Raj[1], Vikramjeet Singh[2], Avneesh Sharma[1], Anupam Das[1], Manodeep Sen[1]

[1] *Department of Microbiology, Dr Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India*
[2] *Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India*

Abstract

Introduction: Bloodstream infections are a leading cause of mortality worldwide, necessitating prompt identification and treatment to improve patient outcomes. Conventional methods are time-consuming, often delaying appropriate antimicrobial therapy. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged as a revolutionary tool for rapid microbial identification. This study aimed to evaluate the utility of direct microbial identification from positive blood cultures using MALDI-TOF MS in reducing diagnostic delays and improving pathogen detection for effective clinical management.

Methodology: This prospective study analyzed 416 monomicrobial positive blood cultures obtained between October 2022 and October 2024 from a tertiary care teaching hospital in North India. After Gram staining and initial processing, direct microbial identification was performed using MALDI-TOF MS. Conventional subculture-based methods were employed as the gold standard for comparison.

Results: Of the 416 isolates, 89% were correctly identified at the species level using the direct method. Among 204 Gram positive cocci, 91.6% were correctly identified, with species-specific rates of 100% for *Staphylococcus aureus* and *Staphylococcus ureilyticus*. Out of 160 Gram negative bacilli, 88.1% were accurately identified, with *Acinetobacter baumannii* showing 97.5% accuracy. *Candida* species showed a correct identification rate of 80.8%, with 100% identification for *Candida albicans* and *Candida krusei*.

Conclusions: Direct microbial identification using MALDI-TOF MS is a rapid, cost-effective alternative to conventional methods, enabling faster pathogen detection and supporting antimicrobial stewardship.

Key words: bacteremia; candidemia; stewardship; diagnostics; septicemia; automation.

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Introduction

Bloodstream infections as a leading source of illness and mortality globally, pose a serious threat to public health. The "gold standard" for diagnosing bloodstream infections remains blood culture (BC), which takes at least 72 hours before a clinician receives the findings of antibiotic susceptibility testing and microbial identification [1]. Timely identification of sepsis is essential, as any delay in administering antibiotics has been shown to raise mortality rates. Each hour of delay in antibiotic treatment in patients of severe sepsis and septic shock increases the risk of mortality by 7.6% [2]. Minimizing turnaround time and providing timely reports to clinicians is essential for enabling prompt administration of effective antimicrobial treatments in sepsis patients, which is crucial for reducing mortality rates [3]. Sepsis is one of the most prevalent adverse events in healthcare facilities, impacting approximately 49 million individuals globally and resulting in 11 million deaths, according to the World Health Organization (WHO) [4].

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized clinical microbiology, particularly in the rapid identification of pathogens directly from positive blood culture bottles [5]. Traditional microbial identification methods, which rely on culture and biochemical analysis, are often time-consuming and may delay the initiation of appropriate therapy. MALDI-TOF MS offers a faster, more accurate alternative, allowing for the identification of microorganisms in a matter of minutes to hours, improving patient outcomes in critical conditions such as bloodstream infections (BSIs) and sepsis [6]. Rapid identification of the causative agent is crucial for guiding antimicrobial therapy, reducing the overuse of broad-spectrum antibiotics, and preventing antimicrobial resistance [7].

Recent advancements in MALDI-TOF MS have demonstrated its capability to directly identify microorganisms from blood culture bottles without the need for subculturing; thus reducing the overall time to diagnosis [8]. Studies have shown that MALDI-TOF MS can accurately identify bacterial species and has been successfully used in combination with other

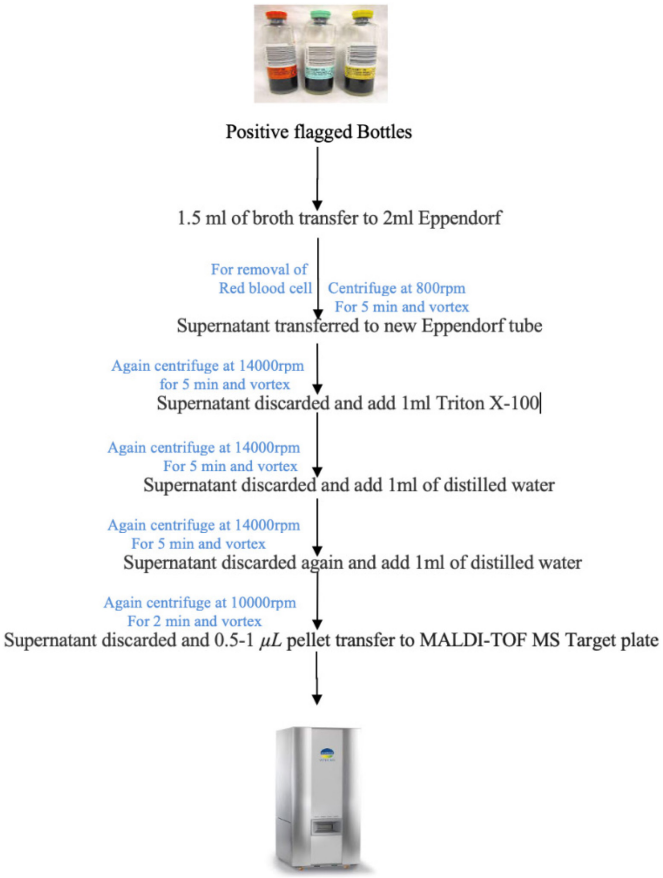
techniques for direct antimicrobial susceptibility testing (AST) and detecting specific resistance mechanisms [9]. The direct detection of pathogens from positive blood cultures has proven effective, even for organisms that are difficult to culture or identify through conventional means [10]. This method holds particular promise in diagnosing infections in immunocompromised patients and those with sepsis, where rapid intervention is essential.

With this background, this study evaluated the utility of direct bacterial identification from positive blood culture bottles using MALDI-TOF MS as a rapid diagnostic approach to reduce the time to pathogen detection and enhance early intervention in cases of bloodstream infections and sepsis.

Methodology

This study analyzed positive flagged aerobic and fungal blood culture bottles from patients admitted to a tertiary care teaching facility of North India, over the period from October 2022 to October 2024. The samples were incubated using the BacT/ALERT 3D system (bioMérieux, Inc., Marcy-l'Étoile, France). Only cultures showing growth of Gram-negative bacteria, Gram-positive bacteria, or yeasts were included; while polymicrobial cultures were excluded from the analysis. This study was approved by the Institutional Review Board / Ethics Committee (Approval # IEC 163/22 RC-460; Approval date 15 September 2022).

Figure 1. Steps of direct identification of blood samples.



Conventional (subculture based) identification method

After the BacT/ALERT 3D system indicated a positive result, the blood culture bottles underwent Gram staining for initial assessment. Subsequently, the samples were subcultured on solid media, including blood agar (Oxoid Ltd., Hampshire, United Kingdom), MacConkey agar (Oxoid Ltd., Hampshire, United Kingdom), and *Candida* differential agar (Oxoid Ltd., Hampshire, United Kingdom) and incubated overnight at 37 °C. On the following day, isolated colonies were prepared for identification by transferring them onto slides. Identification was carried out using the VITEK MS system (bioMérieux, Inc., Marcy-l'Étoile, France), according to the manufacturer's instructions. Identification results were accepted when confidence values ranged from 60% to 99.9%, per the manufacturer's criteria.

Direct microbial identification method

Direct microbial identification was done using the protocol shown in Figure 1. Following Gram staining, the positive blood culture bottle was vigorously shaken to ensure homogeneous mixing. In the case of bacterial isolates, a 1.5 mL sample of the positive blood culture broth was transferred into a 2 mL Eppendorf tube and centrifuged at 800 rpm for 5 minutes to separate red blood cells. The supernatant was transferred to a new Eppendorf tube and centrifuged at 14,000 rpm for 5 minutes. After discarding the supernatant, the resulting pellet was re-suspended in 1 mL of Triton X-100, vortexed, and centrifuged again at 14,000 rpm for 5 minutes. The supernatant was discarded, and the pellet was then re-suspended in 1 mL of distilled water, vortexed, and centrifuged once more at 14,000 rpm for 5 minutes. This process was repeated once more at 10,000 rpm for 2 minutes. After the final centrifugation, the pellet was incubated at room temperature for 15 minutes, and a small amount (0.5–1 µL) of the pellet was spotted onto a MALDI-TOF MS target plate (bioMérieux, Inc., Marcy-l'Étoile, France). Once dried, 1 µL of alpha-cyano-4-hydroxycinnamic acid matrix solution was added to each spot, air-dried, and crystallized at room temperature for 2–4 minutes before MALDI-TOF MS analysis.

In the case of fungal isolates, after following the

same initial steps, the remaining pellet was processed differently. The pellet was mixed with 900 µL of 70% formic acid and centrifuged at 12,000 rpm for 3 minutes. After centrifugation, the supernatant was discarded, and the pellet was vortexed for 30 seconds to ensure thorough mixing. A small amount (0.5–1 µL) of the resulting pellet was then used on the MALDI-TOF MS target plate.

Four-hour plate method

A 4-hour plate method was also applied for fungal isolates to enhance identification accuracy. In this method, a drop of blood culture broth was dispensed onto blood agar and *Candida* differential agar plates and incubated for 4 hours. This short incubation period did not produce discrete colonies but rather a visible thin film on the plate. This film was then scraped using a toothpick and analyzed by VITEK MS (bioMérieux, Inc., Marcy-l'Étoile, France), as previously described.

Statistical analysis

Microbial identification results obtained from the direct microbial identification method were compared to those obtained from the conventional (plate) method and were classified as correct identification, incorrect identification, or no identification. All data were expressed in numbers and percentages. Comparisons of correct identification rates between organisms were analyzed using Fisher's exact test. Separate analyses were performed within Gram-positive, Gram-negative, and yeast groups. Statistical analysis was conducted using GraphPad Prism (GraphPad Software, San Diego, CA, USA), and a p value < 0.05 was considered statistically significant.

Results

A total of 416 blood cultures showing monomicrobial growth were analyzed for direct microbial identification, including 52 fungal isolates, 160 Gram-negative isolates, and 204 Gram-positive isolates. Of 204 Gram-positive isolates, 187 (91.6%) were correctly identified, 15 (7.4%) remained unidentified, and 2 were misidentified, as shown in Table 1. Species-specific correct identification ranged from 50% for *Staphylococcus capitis* to 100% for *Staphylococcus aureus*, *Staphylococcus ureilyticus*, and *Staphylococcus intermedius*. In the case of *Staphylococcus hominis*, 44 of 45 isolates (97.8%) were correctly identified, while *Staphylococcus haemolyticus* had 76 of 86 correctly identified (88.4%), with 8 (9.3%) unidentified and 2 (2.3%) misidentified. *Staphylococcus epidermidis* had 18 of 19 correctly identified (94.7%), and *Enterococcus faecium* and *Enterococcus faecalis* showed 85% and 90.9% correct identifications, respectively. No statistically significant differences were observed in the identification rates

Table 1. Performance characteristics of the direct microbial identification method for the identification of Gram-positive bacteria.

Serial Number	Organism	Isolates (n)	Correct identification	Incorrect identification	No identification	*p* value
1	*E. faecalis*	11	10	0	1	1.0000
2	*E. faecium*	20	17	0	3	0.2237
3	*S. aureus*	11	11	0	0	0.6045
4	*S. capitis*	2	1	0	1	0.1601
5	*S. epidermidis*	19	18	0	1	1.0000
6	*S. haemolyticus*	86	76	2	8	0.1995
7	*S. hominis*	45	44	0	1	0.1276
8	*S. intermedius*	2	2	0	0	1.0000
9	*S. ureilyticus*	8	8	0	0	1.0000
	Total	204	187 (91.6%)	2 (1%)	15 (7.4%)	-

Table 2. Performance characteristics of the direct microbial identification method for the identification of Gram-negative bacteria.

Serial Number	Organism	Isolates (n)	Correct identification	Incorrect identification	No identification	*p* value
1	*A. baumannii*	40	39	0	1	0.0452
2	*A. lwoffii*	4	4	0	0	1.0000
3	*C. koseri*	2	1	1	0	0.2241
4	*E. coli*	16	10	3	3	0.0048
5	*E. meningosepticum*	3	1	0	2	0.0374
6	*K. pneumoniae*	58	51	2	5	1.0000
7	*P. aeruginosa*	11	10	0	1	1.0000
8	*P. rettgeri*	1	0	0	1	0.1188
9	*P. stuartii*	9	9	0	0	0.6007
10	*P. mirabilis*	1	1	0	0	1.0000
11	*S. marcescens*	15	15	0	0	0.2195
	Total	160	141 (88.1%)	6 (3.8%)	13(8.1%)	-

among the different Gram-positive isolates ($p > 0.05$).

Of 160 Gram-negative isolates, 141 (88.1%) were correctly identified, 6 (3.8%) were misidentified, and 13 (8.1%) were not identified as shown in Table 2. Correct identification rates ranged from 33.3% for *Elizabethkingia meningosepticum* to 100% for *Acinetobacter lwoffii*, *Providencia stuartii*, *Proteus mirabilis*, and *Serratia marcescens*. Other isolates had varying identification rates, as follows: *Acinetobacter baumannii* (97.5%), *Klebsiella pneumoniae* (87.9%), *Pseudomonas aeruginosa* (90.9%), and *Escherichia coli* (62.5%). Statistically significant differences in identification rates were observed among Gram-negative isolates ($p < 0.05$), primarily due to lower performance for *Escherichia coli* ($p = 0.0048$) and *Elizabethkingia meningosepticum* ($p = 0.0374$).

Of the 52 *Candida* isolates, 42 (80.8%) were correctly identified, 4 (7.7 %) were misidentified, and 6 (11.5%) were not identified, as shown in Table 3. Correct identification rates ranged from 0% for *Candida rugosa* to 100% for *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, and *Candida pelliculosa*. Other species had varying identification rates, such as *Candida glabrata* (40%) and *Candida tropicalis* (69.2%). In the case of *Candida auris*, the direct method failed to give any identification, so the 4-hour plate method was performed, resulting in 100% correct identification. Statistically significant differences in identification rates were observed among different *Candida* species ($p < 0.05$), primarily due to lower performance for *C. glabrata* ($p = 0.0432$).

Discussion

The direct microbial identification method performed using MALDI-TOF MS yielded 89% correct identification at the species level in 416 monomicrobial growths from positive blood culture bottles. However, the overall performance of these methods varies greatly, with reported data ranging from 60% to 99% concordance at the species level due to different extraction methods used in different studies [11–13]. The challenging and crucial step in the direct MALDI-TOF MS identification process is the removal of blood components from the positive blood culture medium. A variety of chemical buffers, including EDTA, $NaHCO_3$, NH_4Cl, $KHCO_3$, Triton X-100, Tween 80, and saponin, have been used for various sample preparation and extraction processes to remove the blood cells [14].

This study revealed a high concordance rate in identifying Gram-positive cocci (GPC) using MALDI-TOF MS. Of the 204 GPC samples, 187 samples (91.6%) were correctly identified, demonstrating the efficacy of MALDI-TOF MS in the rapid identification of these organisms. *Staphylococcus aureus*, *Staphylococcus ureilyticus*, and *Staphylococcus intermedius* showed 100% correct identification rate, demonstrating the accuracy of the method for these species. *Staphylococcus hominis* had a high identification accuracy of 97.6%, with only 1 sample out of 42 not identified, indicating the reliability of MALDI-TOF MS for this organism. *Enterococcus faecium* and *Enterococcus faecalis* showed reasonably high concordance rates of 85% and 90.9%, respectively. However, a small percentage of samples were not identified, signaling potential limitations in the direct identification of these organisms. Most studies report an overall identification rate of approximately 80% for Gram-positive organisms at the species level, with reported accuracy ranging from 65% to 96% [15–17] Chen *et al.* and Buchan *et al.*, in their studies, reported that the identification rate for *Staphylococcus aureus* exceeded 95% of the isolates tested, whereas for coagulase-negative staphylococci (CoNS) it was below 90% [15,17].

The identification of Gram-negative bacilli (GNB) presented more variability compared to GPC. Among the 160 GNB samples, 141 (88%) were correctly identified. *Klebsiella pneumoniae* was correctly identified in 88% of the samples, *Acinetobacter baumannii* showed a relatively high identification rate of 97.5%. *S. marcescens* was correctly identified in 100% of samples indicating strong performance of

Table 3. Performance characteristics of direct microbial identification method for identification of *Candida species.*

Serial Number	Organism	Isolates (n)	Correct identification	Incorrect identification	No identification	*p* value
1	*C. albicans*	2	2	0	0	1.0000
2	*C. auris*	12	12 *	0	0	0.0924
3	*C. glabrata*	5	2	1	2	0.0432
4	*C. krusei*	1	1	0	0	1.0000
5	*C. parapsilosis*	5	5	0	0	0.5693
6	*C. pelliculosa*	2	2	0	0	1.0000
7	*C. rugosa*	1	0	1	0	0.1923
8	*C. tropicalis*	13	9	2	2	0.2445
9	*C. utilis*	11	9	0	2	1.0000
Total		52	42 (80.8%)	4 (7.7%)	6 (11.5 %)	-

*4 hour plate method.

MALDI-TOF MS for these organisms. *Pseudomonas aeruginosa* was correctly identified in 91% samples, *Citrobacter koseri, E. meningosepticum,* and *P. rettgeri* presented significant challenges, with only 50%, 33%, and 0% correct identification rates respectively. This poor identification rate suggests limitations in the current protocol or the intrinsic characteristics of this organism that make direct identification difficult. Studies have shown that the structural composition of Gram-negative cell walls allows for efficient protein extraction using standard MALDI-TOF MS protocols, resulting in high accuracy with a concordance ranging from 84 to 99% to a species-level identification, with most studies observing > 90% concordance [12,18].

In this study out of 52 *Candida* isolates, 42 (80.8%) were correctly identified; 100% identification rates were observed for *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, and *Candida pelliculosa*; while none of the *Candida rugosa* and *Candida auris* isolates were identified. Previous studies have shown that cell walls of yeast are difficult to disrupt using common MALDI-TOF MS methods, which affects the performance of MALDI-TOF MS identification with identification rates ranging from 0 to 70%. [16,17] An alternative method to identify organisms growing in blood culture bottles is to perform testing on short-incubated colonies—blood culture directly plated to blood agar and incubated at 35 ± 2 °C for 4 hours, to allow for growth of microcolonies [19,20]. These colonies are then treated as normal colony growth with respect to performing MALDI-TOF MS identification. Testing of microcolonies increases turnaround time compared to direct methods, but it removes the need to perform labor-intensive purification [21]. In this study *Candida auris* was not identified by direct identification method, but was correctly identified using this 4-hour plate method, resulting in 100% correct identification

Prolonged empirical therapy, because of delayed reports, has been blamed as a significant barrier to successful antibiotic stewardship programs [22]. Effective antimicrobial stewardship program and patient care depend on the rapid identification of bacteria in blood cultures [23]. Despite widespread consensus that molecular assays are the most effective method for identifying pathogens in blood cultures, their application varies between laboratories across nations, primarily due to the high cost of these tests, the lack of standardized protocols, and the limitation of identifying only a restricted number of species included in the assay panels [24]. Several rapid and cost-effective detection methods for identifying bacterial pathogens like the OmiX-AMP pathogen detection test have been developed; however, they can detect only few pathogens [25]. These limitations can be overcome by using methods such as identifying microorganisms directly from a positive blood culture bottle using the MALDI-TOF MS system with "in-house" techniques or by shortening the incubation time of subculture agar plates to 4 hours for identification using MALDI-TOF MS.

Conclusions

Direct identification from blood cultures using MALDI-TOF MS is more cost-effective and quicker than current molecular methods. While challenges remain for certain organisms like *Citrobacter koseri, E. meningosepticum, P. rettgeri, Candida auris, and C. rugosa;* optimizing protocols and integrating alternative approaches, such as a 4 hour plate method with microcolonies, can enhance identification accuracy and support effective antimicrobial stewardship.

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Authors' contributions

JA, NR, and VS contributed to the development of the core concepts of the study. The study design was formulated by JA and MS. NR was responsible for defining the intellectual content. Literature search was conducted by JA, while data acquisition was carried out by NR and MS. Data analysis was performed by VS. Manuscript preparation was undertaken by JA, and all authors —JA, NR, VS, AS, AD, and MS— contributed to manuscript editing. The manuscript was reviewed by JA, NR, and VS. All authors — JA, NR, VS, AS, AD, and MS — serve as guarantors for the integrity of the work.

Corresponding author

Jyotsna Agarwal, MD.
Department of Microbiology, 3rd Floor, Academic Block, Dr. Ram Manohar Lohia Institute of Medical Sciences,
Vibhuti Khand, Gomti Nagar, Lucknow – 226010, Uttar Pradesh, India
Tel: +91 9415025630
Email: jyotsnaagarwalmicro@gmail.com

Conflict of interest

No conflict of interest is declared.

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