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ORIGINAL ARTICLE

An in-house technique for direct identification of bacteria from positive blood cultures using MALDI-TOF MS

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ABSTRACT

Background: Rapid identification of pathogens from positive blood cultures is crucial for timely antibiotic therapy and effective antimicrobial stewardship. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) enables early organism identification when applied directly to blood culture broth. This study evaluated a simplified, low-cost, in-house direct MALDI-TOF method and compared its diagnostic performance with the standard colony-based approach.

Materials and Methods: This prospective study was conducted at the Microbiology Section of Shaukat Khanum Memorial Cancer Hospital & Research Centre, Lahore, from February to July 2025. One hundred positive blood cultures detected by the BACT/ALERT VIRTUO® system were included. Each specimen underwent both conventional post-culture MALDI-TOF MS identification and an in-house direct method using separator-gel tube centrifugation followed by saline washing. Genus- and species-level concordance between the two methods was calculated.

Results: Overall species-level and genus-level concordance were 60% and 66%, respectively. Gram-negative rods demonstrated the highest species-level accuracy (77.2%), whereas Gram-positive rods showed no species-level identification. Anaerobic bottles achieved higher accuracy (species: 67.3%, genus: 72.7%) than aerobic bottles (species: 51.1%, genus: 57.8%). Excellent agreement was observed for *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Acinetobacter baumannii*, while coagulase-negative staphylococci and *Enterococcus* species exhibited lower identification rates.

Conclusion: The in-house MALDI-TOF method gave rapid, reliable, and cost-effective identification of Gram-negative bacteria, reducing turnaround time. Enhanced performance for Gram-positive identification can be achieved by incorporating detergent-assisted extraction. Integration of direct MALDI-TOF into routine workflows may support quick reporting and targeted therapy.

Keywords: Antimicrobial stewardship, Blood culture, MALDI-TOF MS, Rapid bacterial identification

BACKGROUND

Bloodstream infections (BSIs) are a major cause of morbidity and mortality worldwide. Rapid pathogen identification and early initiation of appropriate therapy are crucial for outcome improvement in patients with sepsis.[1] Rapid microbiological diagnosis also supports effective antimicrobial stewardship and infection control practices.[2]

Although molecular assays provide high diagnostic accuracy for pathogen detection in blood cultures, their use varies widely between countries because of high cost and limited organism coverage. These limitations restrict their routine implementation in many resource-limited laboratories.[2]

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was introduced in the 1980s and became widely adopted in clinical microbiology by 2009. Advances in soft ionization techniques enabled the analysis of large biomolecules, expanding its application from chemical sciences to biological diagnostics. MALDI-TOF MS has significantly improved the speed and accuracy of microbial identification, reducing turnaround time from days to minutes and enabling earlier initiation of appropriate antimicrobial therapy and infection control measures.[3,4]

Direct identification of pathogens from positive blood culture bottles using MALDI-TOF MS has emerged as a valuable rapid diagnostic approach. Several commercial extraction kits are available to remove blood components and mass spectrometry–interfering

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substances, including the VITEK® MS blood culture kit (bioMérieux, Budapest, Hungary), the Sepsityper® kit (Bruker Daltonics, Bremen, Germany), and the fast BACpro® II kit (Nittobo Medical Co., Tokyo, Japan). However, the high cost of these commercial kits limits their routine use in many laboratories, particularly in resource-constrained settings. Consequently, multiple laboratories have developed simplified in-house preparation methods to achieve comparable diagnostic performance at lower cost.[5,6]

This study aimed to develop a simplified in-house direct MALDI-TOF method incorporating centrifugation using a separator-gel tube followed by sequential differential centrifugation. The method was evaluated against the conventional post-culture MALDI-TOF identification approach to assess its performance and determine its suitability for routine use in providing rapid preliminary bacterial identification to clinicians.

MATERIAL AND METHODS

This prospective study was conducted at the Microbiology Department of Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan, between February 2025 and July 2025. Institutional Review Board approval was obtained (IRB number: IRB-23-33), with a waiver of informed consent. A total of 100 blood culture bottles that flagged positive on the BACT/ALERT® VIRTUO® system and demonstrated monomicrobial growth on Gram stain were included. Duplicate samples, polymicrobial cultures, and yeast isolates were excluded.

The sample size was calculated using the standard formula for cross-sectional studies ($n = Z^2p(1-p)/d^2$). A 95% confidence level was used ($Z = 1.96$). In the absence of any local estimate, p-value was conservatively set at 0.50 (margin of error, 10%), and a sample size of 96 was calculated. However, it was rounded to 100 positive blood culture bottles.

A Gram stain was prepared after a blood culture bottle flagged positive. After that, it was inoculated on MacConkey agar, chocolate agar, and blood agar plates and incubated at 35–37 °C for 18–24 hours. After incubation, pure and well-isolated bacterial colonies were subjected to identification by MALDI-TOF MS using the Vitek MS system (bioMérieux, France) according to the manufacturer's instructions.

For the in-house method, five millilitres of blood culture broth demonstrating monomicrobial growth were aspirated into a VACUETTE® Z Serum Sep Clot Activator tube. The tubes were centrifuged at 3,000 rpm for 10 minutes. After discarding the supernatant, the pellet was resuspended in 1 mL of normal saline and transferred to a sterile microcentrifuge tube, followed by centrifugation at 13,000 rpm for 2 minutes. The supernatant was discarded, and the resulting bacterial pellet was used for spotting on MALDI-TOF plate for analysis. Figure-I shows the process of bacterial pellet spotting onto the MALDI target plate for MALDI-TOF MS analysis after the supernatant was removed.

On the MALDI-TOF MS target plate, after drying the spotted colonies using the standard method or the bacterial pellet using the in-house method, 1 µL of α-cyano-4-hydroxycinnamic acid matrix solution was applied to each spot, and then the spots were allowed to air-dry. MALDI-TOF MS analysis was performed using the Vitek MS system (bioMérieux, France), with spectra acquisition and identification interpreted according to the manufacturer's validated database and confidence thresholds. For this study, we used SPSS 24.0, developed by IBM Corp. of Armonk, NY, USA, to examine the data collected. Frequencies and percentages were used to indicate descriptive statistics.

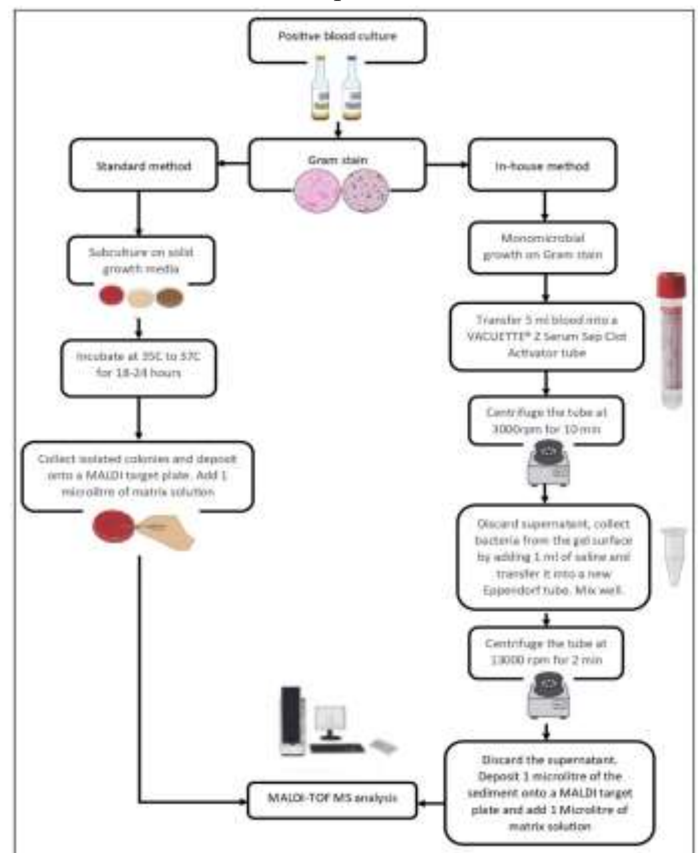

Figure-I: Schematic representation of the workflow followed in the study

RESULTS

A total of 100 positive blood culture bottles were included in the analysis. Direct MALDI-TOF detection method had a 60% overall species-level concordance and a 66% genus-level concordance with the conventional colony-based approach, suggesting a moderate agreement and the correct identification of approximately two-thirds of the isolates.

Direct MALDI-TOF identification performance differed significantly between different Gram groups (Figure 2). The most accurately identified organisms were Gram-negative rods (GNR), whereas the success rate of Gram-positive organisms, especially rods, was low. Out of 58 GNR, 81% were placed in the proper species, 82.7% in the correct genus, and 17.2% yielded no identification. The 95% confidence intervals for these percentages were 0.65 to 0.86 and 0.69 to 0.89, respectively. With 12 Gram-positive cocci chains (GPC chains), the species-level concordance rate was 41.7%, the genus-level concordance rate was 66.7% (95% CI: 0.19-0.68) and the no-identification result rate was 25%. Out of 25 Gram-positive cocci in clusters, 52% were able to be identified at the species level and 68% at the genus level (95% CI: 0.26-0.61 and 0.29-0.65); however, 32% of these samples could not be identified. Gram-positive rods (GPR, n = 5) demonstrated poor performance, with no species-level concordance, only one isolate (20%) achieving genus-level identification (95% CI: 0.04–0.62), and three isolates (60%) yielding no identification.

Anaerobic bottles (n = 55) performed better than aerobic bottles (n = 45) when comparing bottle types, achieving 72.7% against 57.8% genus-level accuracy and 67.3% versus 51.1% species-level concordance. Twenty-three (51.1%) and 26 (57.8%) isolates from aerobic bottles (A, n = 45) were properly identified at the species and genus levels, respectively. Fifteen bottles (33.3%) did not provide a direct identification. Anaerobic bottles (An, n = 55) performed better in contrast, with 40 isolates (72.7%) successfully matching to the genus level and 37 (67.3%) correctly matching to the species level. Twelve (21.8%) bottles were not identified by the direct method. With almost one-third fewer identification errors than aerobic bottles, anaerobic bottles produced better accuracy overall at the species and genus levels, indicating that improved protein extraction and spectral quality may be achieved by increased bacterial biomass or more advantageous matrix conditions in anaerobic conditions.

Different species performed differently in the organism-wise accuracy of direct MALDI-TOF identification, with generally stronger concordance among GNR compared to GPC (Table 1).

Overall, Gram-negative bacteria achieved the highest species-level accuracy. *Proteus mirabilis*, *Acinetobacter baumannii*, *Enterobacter hormaechei*, *Enterobacter cloacae*, *Citrobacter koseri*, *Pseudomonas aeruginosa*, *and Klebsiella pneumoniae* demonstrated 100% species-level concordance. *Salmonella enterica* and *Escherichia coli* showed species-level concordance of 67% and 81%, respectively. Among Gram-positive organisms, *Staphylococcus aureus* achieved 80% species-level concordance, whereas coagulase-negative staphylococci frequently yielded no-identification results. *Enterococcus faecium* demonstrated moderate genus-level concordance (71.4%), while *Listeria monocytogenes* was not successfully identified. These findings support the utility of direct MALDI-TOF for Gram-negative bacteremia while emphasizing the need for confirmatory testing for Gram-positive organisms.

The moderate species-level (60%) and genus-level (66%) concordance indicates that direct MALDI-TOF serves best as an adjunct rather than a replacement for conventional culture-based identification. Performance was highest for GNRs and lowest for GPRs. A broad range of confidence intervals observed in small subgroups reflects limited sample size and warrants cautious interpretation.

Table-I: Comparing the in-house MALDI-TOF MS approach with the conventional culture-dependent method for organism identification.

Isolate ID by the standard method	Total no. of isolates	Genus-level matched ID		Species-level matched ID		Unidentified isolates		Misidentified isolated	
		(n)	%	(n)	%	(n)	%	(n)	%
Gram-Negative Bacilli Group (13 isolate types)									
Escherichia coli	21	17	80.9	17	80.9	4	19.1	0	0.0
Salmonella enterica	15	10	66.7	10	66.7	5	33.3	0	0.0
Klebsiella pneumoniae	4	4	100.0	4	100.0	0	0.0	0	0.0

Klebsiella oxytoca	1	0	0.0	0	0.0	1	100.0	0	0.0
Pseudomonas aeruginosa	3	3	100.0	3	100.0	0	0.0	0	0.0
Acinetobacter baumannii	2	2	100.0	2	100.0	0	0.0	0	0.
Haemophilus influenzae	1	1	100.0	0	0.0	0	0.0	0	0.0
Proteus mirabilis	2	2	100.0	2	100.0	0	0.0	0	0.0
Proteus vulgaris	3	3	100.0	3	100.0	0	0.0	0	0.0
Enterobacter cloacae	3	3	100.0	3	100.0	0	0.0	0	0.0
Enterobacter hormaechei	1	1	100.0	1	100.0	0	0.0	0	0.0
Aeromonas hydrophila	1	1	100.0	1	100.0	0	0.0	0	0.0
Citrobacter koseri	1	1	100.0	1	100.0	0	0.0	0	0.0
Gram-Positive Cocci in Clusters (5 isolate types)									
Staphylococcus hominis	9	6	66.7	5	55.6	3	33.3	0	0.0
Staphylococcus haemolyticus	3	2	66.6	1	33.3	1	33.3	0	0.0
Staphylococcus aureus	5	4	80.0	4	80.0	1	20.0	0	0.0
Staphylococcus epidermidis	7	5	71.4	3	42.8	2	28.5	0	0.0
Staphylococcus capitis	1	0	0.0	0	0.0	1	100.0	0	0.0
Gram-Positive Cocci in Chains (5 isolate types)									
Enterococcus faecium	7	5	71.4	3	42.8	2	28.5	0	0.0
Streptococcus anginosus	1	0	0.0	0	0.0	1	100.0	0	0.0
Streptococcus pneumoniae	2	2	100.0	1	50.0	0	0.0	0	0.0
Streptococcus pyogenes	1	1	100.0	1	100.0	0	0.0	0	0.0
Streptococcus salivarius	1	1	100.0	0	0.0	0	0.0	0	0.0
Gram-Positive Rods (3 isolate types)									
Listeria monocytogenes	2	0	0.0	0	0.0	2	100.0	0	0.0
Corynebacterium amycolatum	2	1	50.0	0	0.0	1	50.0	0	0.0
Corynebacterium jeikeium	1	0	0.0	0	0.0	0	0.0	1	100.0

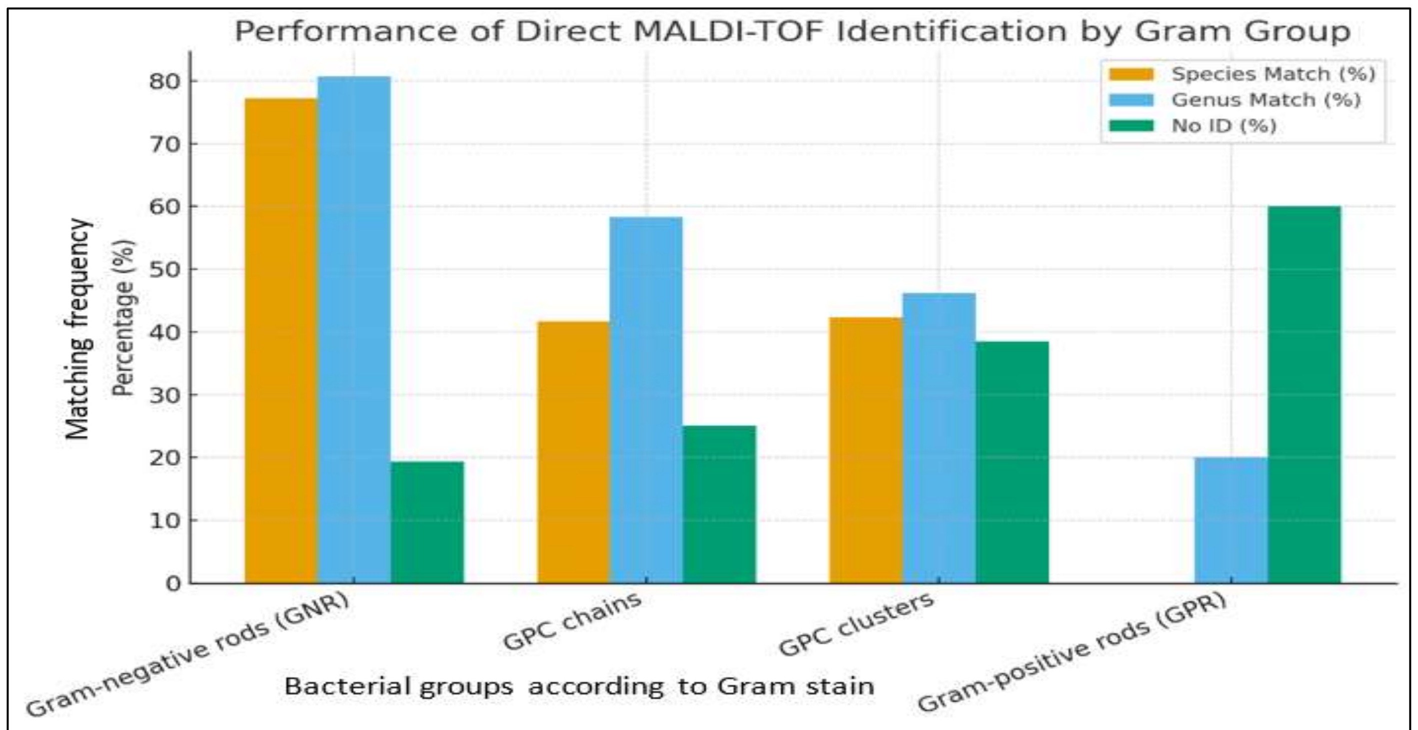

Figure-2: Overview of precise identification using the in-house method across various gram groups.

DISCUSSION

This study has shown a moderate overall agreement of direct MALDI-TOF identification from positive blood culture broth over the conventional culture plate identification, with superior performance in GNR than in Gram-positive organisms. These patterns are consistent with previously published literature and highlight the role of MALDI-TOF as a rapid supportive diagnostic tool instead of a replacement for conventional colony-based method.

Multiple studies report that direct identification from positive BCs is more successful for GNR and less

vigorous for Gram-positives due to thicker cell walls, reduced protein yield efficiency, and interference with the matrix. A higher species-level identification for Enterobacterales and non-fermenters has been observed in multiple reviews and evaluations when compared to staphylococci and streptococci, which matches our gradient (GNR > GPC > GPR).[7-11] Different protocols have reported variable and a broad range of concordance rates, dependent on sample processing methodologies, extraction procedures, and instrument thresholds, highlighting the inherent variability of direct proteomic identification.[4, 12-14]

In our study, higher identification rate was observed from anaerobic bottles when compared with aerobic bottles. Factors responsible for enhanced protein extraction from anaerobic bottles include great organism biomass at the time of positivity, improved spectral quality and favorable matrix composition. Ko *et al*. compared an in-house microfiltration protocol to Sepsityper and showed an increased overall accuracy, with particularly significant outcomes for anaerobes, validating our findings of outperformance of anaerobic bottles when compared to aerobic counterparts.[7] Our organism-class hierarchy (GNR > Gram-positives > yeasts), especially from anaerobic bottles, has also been reinforced by Dai *et al*., who achieved 87.6% identification at a score ≥1.7 with the same pattern.[12]

A practical clinical implication of failure of direct MALDI-TOF identification in suspected staphylococcal cases is skewing of failed IDs toward CoNS rather than *S. aureus*. Hamilton *et al*. observed a higher failure rate for coagulase-negative staphylococci when compared with *S. aureus* in a post-hoc analysis of the RAPIDO trial, which suggests that cautious de-escalation strategies can be considered in selected low-risk cases while awaiting definitive identification.[8]

Although our study focused on monomicrobial blood cultures, the real-world labs encounter polymicrobial bloodstream infections, which is a significant limitation for direct MALDI-TOF identification due to mixed and overlapping spectra of different microorganisms. It has been previously observed that mixed genera are only identified in approximately one-third of mixed broth cultures. Gray *et al.* explained that detection of mixed cultures can be improved by new, improvised versions of software in the future.[15] Almuhayawi *et al*. showed that molecular platforms like Film Array can help in resolving this problem. This method identified 92% of isolates and fully resolved 80% of polymicrobial bottles, as compared to direct MALDI-TOF methodology, which identified only 28% of isolates.[14] This highlights the requirement for adjunct molecular testing or conventional subculturing when there is suspicion of mixed, polymicrobial infection.

The methodology involved in sample preparation plays a key role in direct identification. Multiple studies show that incorporation of detergent-assisted lysis or ethanol/formic extraction during processing can improve Gram-positive identification. Yoo *et al*. improved direct identification and AST concordance by adding 0.1% SDS lysis to SepsiPrep across BD BACTEC and BacT/Alert systems.[9] Karadağ *et al*. found SDS lysis superior to Sepsityper and centrifugation–protein extraction (74.2% vs 55.0% species-level accuracy), cautioning against relaxed confidence cutoffs.[13] Dai *et al*. achieved 87.6% overall ID in 10–20 min at minimal cost by combining density centrifugation with chemical lysis.[12] Similarly, Wang *et al*., while using a local Bioyong kit, demonstrated an increased accuracy and reinforced that a low-cost, optimized in-house workflow can compete with commercial kits.[1]

Early organism identification has multiple clinical advantages, including reduced TAT, early optimization of therapy, and improved stewardship. Zengin Canalp & Bayraktar demonstrated that direct identification cut TAT to ~2.9 hours, versus ~19.5 hours with colony ID.[2] Puckett *et al*. and Pérez-López *et al*. showed faster organism ID reduced time to effective/optimal therapy and lowered admissions for contaminant cultures.[4,11] In hematologic malignancies, Watanabe *et al*. reported acceptable direct identification with Sepsityper, thus providing clinically actionable results and reduced turnaround time in a high-risk population.[10] Kuo *et al*. and Noll *et al*. illustrated the performance of the direct MALDI-TOF ID method in cases of PJIs, where synovial fluid, inoculated into blood culture bottles, substantially shortened the TAT for identification with high concordance, especially for GNRs.[16, 17]

The findings of this study have practical implications for both interpretation and workflow optimization. In routine laboratory practice, direct MALDI-TOF detection may be prioritized for GNRs to facilitate early antimicrobial optimization. However, for failed staphylococcal IDs, which more likely indicate coagulase-negative staphylococci than *S. aureus*,

conventional subculture-based identification should remain the primary confirmatory method. Protocols involving pretreatment optimization, as performed by Yoo, Karadağ, and Dai, may further increase the yield of Gram-positive organisms without substantially increasing TAT.[9, 12, 13]

However, there were several limitations. This was a single-center study with a high proportion of Gram-negative isolates, limiting generalizability. Although a turnaround time or formal cost assessment was not performed, the processing required less than 30 minutes. Exclusion of polymicrobial and yeast-containing cultures likely overestimates performance, and small subgroup sizes for certain Gram-positive species limit statistical precision.

CONCLUSION

The in-house direct MALDI-TOF protocol achieved reliable identification for most Gram-negative isolates, with moderate success for Gram-positive organisms, and better yield from anaerobic bottles. It provides a practical and low-cost alternative for the early detection of bloodstream pathogens in clinical laboratories. Further optimization, particularly for Gram-positive coverage through detergent-assisted extraction, could enhance its clinical utility.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Nida Safdar: Acquisition, analysis, and interpretation of data, manuscript writing, critical revisions, final approval, accountable for all aspects of publication.

Aqib Sultan: Acquisition and analysis, of data, final approval, accountable for all aspects of publication

Nasrullah Malik: Critical revisions, final approval, accountable for all aspects of publication

Saira Saleem: Manuscript writing, critical revisions, final approval, accountable for all aspects of publication.

Summiya Nizamuddin: Study conception, manuscript writing, critical revisions, final approval, accountable for all aspects of publication.

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