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Broad-Spectrum Bacterial Identification by MALDI-TOF MS in Routine Clinical Microbiology: A Laboratory-Based Study from Western Rajasthan

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Abstract

Background & Objectives: Rapid bacterial identification facilitates timely clinical decision-making, infection prevention and antimicrobial stewardship. Although matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is established for microbial identification, its practical value in routine laboratories is particularly relevant for diverse, non-fermenting and uncommon organisms. This study evaluated the real-world utility of MALDI-TOF MS for species-level identification of clinically significant bacterial isolates and compared its operational workflow with conventional biochemical identification.

Methods: A retrospective observational study was conducted from January 2024 to March 2025 in the Microbiology Department of a tertiary-care teaching hospital and research centre in Western Rajasthan, India. A total of 1,000 consecutive, non-duplicate, clinically significant bacterial isolates were analysed. Conventional identification involved colony morphology, Gram staining and biochemical reactions, while all isolates were identified using the VITEK® MS PRIME MALDI-TOF MS system. Bacterial distribution, species-level identification, uncommon organisms, turnaround time and technician hands-on time were assessed.

Results: Among 1,000 isolates, 631 (63.1%) were Gram-negative bacilli and 369 (36.9%) Gram-positive cocci. Fermentative and non-fermentative Gram-negative bacilli comprised 287 and 344 isolates, respectively. Escherichia coli predominated among fermenters (131; 45.6%), Acinetobacter spp. among non-fermenters (189; 54.9%), and Staphylococcus aureus among Gram-positive cocci (169; 45.8%). MALDI-TOF MS differentiated multiple Acinetobacter, Pseudomonas and coagulase-negative Staphylococcus species and identified several uncommon organisms to species level. Identification required 5–15 min versus 24–48 h by conventional methods, with hands-on time of 3–5 versus 20–30 min.

Conclusion: MALDI-TOF MS provides rapid species-level identification across diverse bacterial isolates and

has particular practical value for non-fermenters, coagulase-negative Staphylococcus and uncommon organisms in routine clinical microbiology.

Keywords: MALDI-TOF MS, Non-Fermenting Gram-Negative Bacilli, Uncommon Bacteria, Species-Level Identification.

Introduction

Accurate identification of bacterial pathogens is a fundamental function of the clinical microbiology laboratory. In tertiary-care hospitals, laboratories encounter a wide range of organisms, including common pathogens, healthcare-associated pathogens, multidrug-resistant organisms and relatively uncommon bacteria. Timely and accurate identification can assist clinical decision-making, infection prevention and control, and diagnostic stewardship [1–3.]

Conventional bacterial identification remains dependent on colony morphology, Gram staining and biochemical reactions. These methods are inexpensive, familiar and widely applicable but may require several sequential tests and prolonged incubation. Species-level identification may consequently take 24–48 h after adequate colony growth, and identification of uncommon or phenotypically similar organisms can be challenging [2,5,7]

MALDI-TOF MS has transformed routine microbial identification by replacing much of the conventional biochemical approach with protein spectral profiling[1,2,7,14]. A characteristic protein spectrum generated from a bacterial colony is compared with reference spectra contained in the instrument database, allowing rapid identification once an adequate colony is available[2,11,14]. The technology has consequently become an important component of modern clinical microbiology laboratories[1,2,14].

Previous investigations have established the rapidity and broad utility of MALDI-TOF MS in routine clinical microbiology[1,2,11,14]. Its role has been particularly relevant for non-fermenting Gram-negative bacilli, for which conventional phenotypic identification may be difficult because of overlapping biochemical characteristics[4,10]. MALDI-TOF MS has also demonstrated utility in the identification of uncommon and difficult-to-identify microorganisms[8,12,14,17] and has been applied in hospital infection-control and public-health investigations[12,13].

However, the value of MALDI-TOF MS in laboratory-based studies lies in demonstrating how the technology performs in specific routine diagnostic settings, particularly where there is a diverse bacterial spectrum and a substantial proportion of organisms that are difficult to resolve phenotypically. Local laboratory experience can also provide information relevant to diagnostic workflow and implementation[1,14]. Rapid identification may support diagnostic and antimicrobial stewardship when integrated with antimicrobial susceptibility testing and communication of microbiological results to clinicians [3,15].

Data describing routine implementation of MALDI-TOF MS in tertiary-care laboratories in Western Rajasthan remain limited. The present study was therefore undertaken to evaluate the spectrum of clinically significant bacterial isolates identified using MALDI-TOF MS and to compare its operational performance with conventional biochemical identification, with particular emphasis on identification turnaround time, technician hands-on time, species-level identification and uncommon bacterial isolates.

Materials and Methods

Study design and setting- A retrospective observational study was conducted in the Department of Microbiology in a tertiary care teaching hospital and research centre in Western Rajasthan, India.

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Study period - Laboratory records generated between January 2024 and March 2025 were reviewed.

Study population- A total of 1,000 consecutive, non-duplicate, clinically significant bacterial isolates recovered from routine clinical specimens were included. Specimens comprised blood, urine, pus, sputum, endotracheal aspirates, bronchoalveolar lavage, cerebrospinal fluid, pleural fluid and other sterile body fluids (synovial fluid and peritoneal fluid). Clinically significant bacterial isolates with adequate growth for identification were included. Duplicate isolates from the same patient, environmental isolates, unresolved mixed cultures and clinically insignificant contaminants were excluded.

Conventional identification- Specimens were processed according to standard microbiological procedures and inoculated onto appropriate culture media, including blood agar and MacConkey agar. Cultures were incubated at 35–37°C for 18–24 h, as appropriate. Blood cultures were processed using the BACT/ALERT® system. Conventional identification was based on colony morphology, Gram staining and appropriate biochemical tests, including catalase, oxidase and organism-specific reactions. The identification interval was approximately 24–48 h after visible colony growth.

MALDI-TOF MS identification

All isolates were identified using the VITEK® MS PRIME MALDI-TOF MS system (bioMérieux) according to the manufacturer's instructions. A fresh, well-isolated colony obtained after 18–24 h incubation was transferred onto a disposable VITEK® MS target slide. One microlitre of α-cyano-4-hydroxycinnamic acid matrix was applied and allowed to dry before analysis.

The protein spectral profile generated from each isolate was compared automatically with the manufacturer's reference database. Results were interpreted according to the manufacturer's recommended confidence criteria. Escherichia coli ATCC 8739 was used as the control strain.

Primary objective: To assess the identification turnaround time following availability of a suitable colony. Secondary objectives included assessment of bacterial distribution according to specimen type, species-level identification, identification of uncommon organisms, and technician hands-on time.

Statistical Analysis: Data were entered into Microsoft Excel and analysed using appropriate statistical methods. Categorical variables were expressed as frequencies and percentages. Associations between major bacterial groups and clinical specimen type were assessed using the Pearson chi-square test. A P value <0.05 was considered statistically significant.

Results

A total of 1,000 consecutive, non-duplicate clinically significant bacterial isolates were included. Gram-negative bacilli (GNB) accounted for 631 (63.1%) isolates, comprising 287 (28.7%) fermentative Gram-negative bacilli and 344 (34.4%) Non Fermentative GNB(NFGNB). Gram-positive cocci (GPC) accounted for 369 (36.9%) isolates.

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Table 1:

Samples	GNB		GPC	Total
	Fermenters	Non-fermenters		
Pus	102 (26.35%)	88 (22.74%)	197 (50.90%)	387 (38.70%)
Blood	47 (24.35%)	66 (34.20%)	80 (41.45%)	193 (19.30)
Urine	74 (48.05%)	38 (24.67%)	42 (27.27%)	154 (15.40%)
Sputum/ETA	52 (23.52%)	124 (56.11%)	45 (20.36%)	221 (22.10%)
BAL	05 (29.41%)	10 (58.82%)	02 (11.76%)	17 (1.7%)
Pleural Fluid	03 (33.33%)	05 (55.55%)	01 (11.11%)	09 (0.9%)
CSF	04 (26.66%)	09 (60%)	02 (13.33)	15 (1.5%)
Others	00	04 (100%)	00	04 (0.4%)
Total	287 (28.70%)	344(34.40%)	369 (36.90%)	1000

GNB (Gram-negative bacilli); GPC (Gram-positive cocci); ETA (endotracheal aspirate); BAL (bronchoalveolar lavage); CSF (cerebrospinal fluid).

*Other: synovial fluid and peritoneal fluid.

Note: Percentages represent the distribution of bacterial groups within each specimen type.

Pus was the predominant specimen source, accounting for 387 (38.7%) isolates, followed by sputum/endotracheal aspirates (221; 22.1%), blood (193; 19.3%) and urine (154; 15.4%). NFGNB represented a substantial proportion of respiratory isolates, whereas Gram-positive cocci predominated among isolates from pus. The association between bacterial group and clinical specimen was statistically significant (Pearson χ^2=137.36, P<0.001) (Table 1)

Table 2: Distribution of fermentative Gram-negative bacilli according to clinical specimen.

Organism	Total, n (%)	Pus	Blood	Urine	Sputum/ETA	BAL	Pleural fluid	CSF
Escherichia coli	131 (45.6)	38	25	37	23	3	2	3
Klebsiella spp.	64 (22.3)	21	9	16	15	1	1	1
Enterobacter spp.	18 (6.3)	12	3	1	2	–	–	–
Citrobacter spp.	15 (5.2)	6	3	5	1	–	–	–
Salmonella enterica	4 (1.4)	–	4	–	–	–	–	–
Brucella intermedia	1 (0.3)	–	1	–	–	–	–	–
Serratia marcescens	7 (2.4)	2	–	–	4	1	–	–
Proteus spp.	31 (10.8)	15	–	16	–	–	–	–
Providencia spp.	11 (3.8)	5	–	–	6	–	–	–
Morganella morganii	5 (1.7)	3	–	2	–	–	–	–
Total	287 (100)	102	45	61	51	5	3	4

ETA: endotracheal aspirate; **BAL:** bronchoalveolar lavage; **CSF:** cerebrospinal fluid.

Among 287 fermentative Gram-negative bacilli, Escherichia coli was predominant (131; 45.6%), followed

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by Klebsiella spp. (64; 22.3%). Species-level identification demonstrated diversity within these groups, including K. pneumoniae and K. aerogenes; E. cloacae, E. hormaechei and E. bugandensis; C. freundii, C. koseri and C. braakii; and P. mirabilis, P. vulgaris and P. pinneri. Providencia spp. and Morganella morganii were also identified. The specimen-wise distribution of fermentative GNB is shown in Table 2.

Table 3: Distribution of non-fermentative Gram-negative bacilli according to clinical specimen

Isolates	Total n (%)	Pus	Blood	Urine	Sputum/ETA	BAL	Pleural fluid	CSF	Other
Pseudomonas spp.	118 (34.3)	29	9	22	50	4	1	–	2
Acinetobacter spp.	189 (54.9)	43	47	13	67	5	3	9	2
Stenotrophomonas maltophilia	25 (7.3)	13	3	1	7	1	–	–	–
Ralstonia pickettii	6 (1.7)	–	6	–	–	–	–	–	–
Burkholderia cepacia complex	3 (0.9)	3	–	–	–	–	–	–	–
Oligella urethralis	2 (0.6)	–	–	2	–	–	–	–	–
Chryseobacterium indologenes	1 (0.3)	–	1	–	–	–	–	–	–
Total	344 (100)	88	66	38	124	10	4	9	4

ETA: endotracheal aspirate; **BAL:** bronchoalveolar lavage; **CSF:** cerebrospinal fluid.

Among the 344 NFGNB, Acinetobacter spp. were the predominant group, accounting for 189 (54.9%) isolates, followed by Pseudomonas spp. [118 (34.3%)] and Stenotrophomonas maltophilia [25 (7.3%)]. Among the 189 Acinetobacter isolates, A. baumannii was the predominant species [107 (56.6%)], followed by A. lwoffii [27 (14.3%)], A. radioresistens [20 (10.6%)], A. junii [19 (10.1%)], A. schindleri [14 (7.4%)] and A. bereziniae [2 (1.1%)]. Out of 118 Pseudomonas isolates, P. aeruginosa was predominant, accounting for 91 (77.1%) isolates, followed by P. stutzeri [13 (11.0%)], P. fluorescens [12 (10.2%)] and P. putida [2 (1.7%)]. The detailed specimen-wise distribution of NFGNB and their species-level identification is presented in Table 3.

Table 4: Distribution of Gram-positive cocci according to clinical specimen

Isolates	Total, n (%)	Pus	Blood	Urine	Sputum/ETA	BAL	Pleural fluid	CSF
Staphylococcus aureus	169 (45.8)	92	30	23	22	1	1	–
Staphylococcus lugdunensis	2 (0.5)	2	–	–	–	–	–	–
CONS	123 (33.3)	60	42	3	20	1	–	–
Streptococcus pneumoniae	1 (0.3)	–	1	–	–	–	–	–
Enterococcus spp.	74 (20.1)	46	7	16	3	–	–	2
Total	369 (100)	200	80	42	45	2	1	2

ETA: endotracheal aspirate; **BAL:** bronchoalveolar lavage; **CSF:** cerebrospinal fluid.

Among the 369 GPC, Staphylococcus aureus was the predominant organism [169 (45.8%)], followed by coagulase-negative Staphylococcus (CONS) [123

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(33.3%)] and Enterococcus spp. [74 (20.1%)]. A single isolate of Streptococcus pneumoniae (0.3%) was identified. Among the 123 CONS isolates, species-level identification demonstrated S. epidermidis [54 (43.9%)], S. haemolyticus [47 (38.2%)], S. hominis [15 (12.2%)], S. chromogenes [3 (2.4%)], S. capitis [2 (1.6%)] and S. gallinarum [2 (1.6%)]. Among the 74 Enterococcus isolates, E. faecalis accounted for 39 (52.7%) and E. faecium for 35 (47.3%). The specimen-wise distribution of GPC and species-level identification is presented in Table 4.

MALDI-TOF MS enabled species-level identification of several uncommon isolates that were reported only as broad groups by conventional methods. These included Achromobacter xylosoxidans, Burkholderia cenocepacia, B. contaminans, Mammaliicoccus sciuri, Corynebacterium striatum, Leclercia adecarboxylata, Rothia mucilaginosa, Rothia kristinae, Haemophilus parainfluenzae, Eikenella corrodens, Vibrio fluvialis, and Nocardia neocaledoniensis. Following availability of a suitable colony, MALDI-TOF MS required approximately 5–15 min for identification, whereas conventional biochemical identification required approximately 24–48 h. MALDI-TOF MS markedly shortened the identification time after colony growth to 5–15 min compared with 24–48 h by conventional methods, while reducing technician hands-on time from 20–30 min to 3–5 min. It also provided rapid species-level identification, including for difficult and uncommon organisms that were frequently identified only to broader taxonomic levels by conventional methods.

Discussion

The present study provides an assessment of MALDI-TOF MS in routine clinical bacteriology based on 1,000 consecutive clinical bacterial isolates. Rather than evaluating the technology solely as a new diagnostic platform, the study highlights its practical role in providing rapid species-level information across a heterogeneous population of clinical isolates [1,2,14].

The major operational advantage was the marked reduction in the identification interval following colony growth. MALDI-TOF MS generated identification within approximately 5–15 min compared with 24–48 h using conventional biochemical methods. This observation is consistent with previous reports describing MALDI-TOF MS as a rapid alternative to sequential biochemical testing [1,2,5,11,14].

The importance of this reduction lies not simply in laboratory convenience. Earlier species-level identification can provide clinicians with more specific microbiological information while antimicrobial susceptibility testing is being completed. Such information may support diagnostic decision-making and complement antimicrobial stewardship when integrated appropriately into the clinical workflow [3,15].

An important feature of the present isolate population was the substantial representation of NFGNB. *Acinetobacter* and *Pseudomonas* are important healthcare-associated organisms and frequently demonstrate clinically significant antimicrobial resistance[9,10]. Conventional phenotypic identification of NFGNB can be challenging because several species share overlapping biochemical characteristics [4,10]. Gautam et al. specifically demonstrated the value of MALDI-TOF MS for identification of NFGNB [4]. The ability of the present system to differentiate multiple Acinetobacter and Pseudomonas species therefore represents an important practical advantage[4,10].

The finding is particularly relevant because reporting an isolate only as an *Acinetobacter* or *Pseudomonas* species group may provide less information than species-level identification. Species-level resolution can improve the

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microbiological characterization of isolates and may assist surveillance and interpretation of clinically significant organisms, particularly when combined with standardized antimicrobial susceptibility testing [6,10,13].

The identification of CONS to species level represents another useful application. CONS are commonly recovered in clinical microbiology laboratories and may represent either colonization or true infection. Species-level information can be useful in selected clinical settings, particularly bloodstream and device-associated infections, although microbiological identification must always be interpreted together with the clinical picture and blood-culture findings [1,2,14].

A distinctive aspect of this study was the identification of uncommon and difficult-to-identify organisms. Achromobacter, Burkholderia, Rothia, Leclercia, Eikenella, Nocardia and Mammaliicoccus were among the organisms identified to species level. Conventional biochemical methods may provide only a broad identification for such isolates, particularly when the organism is infrequently encountered or has atypical biochemical characteristics [8,14,17].

MALDI-TOF MS has been recognised as useful for uncommon organisms and has also been applied in hospital infection-control investigations[8,12,13]. In this context, the value of the technology is not restricted to faster identification of common organisms. Its ability to provide higher taxonomic resolution across a diverse isolate population may be particularly relevant to laboratories serving tertiary-care hospitals [1,8,12,14].

The findings also have relevance to laboratory-based antimicrobial-resistance surveillance. Reliable species-level identification is an important component of organism-specific surveillance, and rapid identification can strengthen the diagnostic component of antimicrobial-resistance monitoring when combined with standardized susceptibility testing[6,10]. However, the present study did not directly evaluate the performance of MALDI-TOF MS within an external AMR surveillance network.

The reduction in technician hands-on time observed in this study is also relevant to laboratory workflow. Conventional identification can involve several individual biochemical tests and repeated handling of isolates. MALDI-TOF MS reduces many of these steps to colony transfer, matrix application and spectral analysis [1,2,14]. In a high-volume laboratory, this can potentially allow microbiologists and technical staff to devote more time to antimicrobial susceptibility testing, quality assurance, result interpretation and other diagnostic activities [1,14].

The study has several strengths. It included a relatively large series of 1,000 consecutive non-duplicate clinical isolates rather than a selected collection of reference strains. The bacterial population represented common pathogens as well as NFGNB, CONS and uncommon organisms. This provided a diverse representation of the bacterial profile encountered in a tertiary-care clinical microbiology laboratory.

The study also has limitations. It was retrospective, data based and single-centre study, limiting generalisability. Turnaround time was measured from availability of a suitable colony and therefore does not represent the complete specimen-to-report interval. The study did not prospectively evaluate antibiotic de-escalation, antimicrobial consumption, patient outcomes or cost-effectiveness. Furthermore, because conventional identification and MALDI-TOF MS were not independently compared against a reference identification method for all isolates, the study was designed primarily as an operational workflow

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assessment rather than a formal diagnostic accuracy study.

Conclusion

MALDI-TOF MS provided rapid identification after colony growth while requiring substantially less technician hands-on time than conventional biochemical methods. The system provided species-level identification among GNB and GPC and facilitated identification of several uncommon organisms that had received broader phenotypic identification. MALDI-TOF MS should therefore be regarded as a valuable component of clinical microbiology rather than simply a replacement for conventional biochemical testing. Conventional methods and appropriate confirmatory techniques remain essential for unusual, uncertain or poorly represented organisms. Integration of MALDI-TOF MS with antimicrobial susceptibility testing, laboratory quality systems and clinical communication may further enhance its contribution to diagnostic stewardship and AMR surveillance.

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