VER
Vascular & Endovascular Review

Diagnostic Approaches for Detecting Metallo-β-Lactamase–Producing Gram-Negative Bacteria: A Review of MALDI-TOF Mass Spectrometry, Culture Methods, and Emerging Machine Learning Applications

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ABSTRACT

Metallo-β-lactamase (MBL)–producing Gram-negative bacteria represent a major challenge to global healthcare due to their ability to confer resistance to carbapenems, often leaving limited therapeutic options. Early and accurate detection of these organisms is essential for appropriate antimicrobial therapy, infection control, and antimicrobial stewardship. Conventional culture-based and phenotypic methods remain widely used but are limited by prolonged turnaround times, subjective interpretation, and variable diagnostic performance. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a rapid tool for bacterial identification and has recently been adapted for functional detection of antimicrobial resistance through modified carbapenem hydrolysis assays. In parallel, machine learning approaches applied to MALDI-TOF spectral data have shown promising results in automating and enhancing resistance detection. This review critically summarizes current diagnostic approaches for MBL detection, compares their diagnostic performance, and discusses the evolving role of machine learning–assisted MALDI-TOF MS in routine clinical microbiology. Key challenges, standardization issues, and future research directions are also highlighted to support clinical translation.

KEYWORDS: Metallo-β-lactamase; MALDI-TOF MS; Gram-negative bacteria; Carbapenem resistance; Machine learning; Diagnostic accuracy.

How to Cite: Sangita Chanda, Dr. Dhruba Hari Chandi, Dr. Utkarsha Pacharaney, Ajay Indrale, (2026) Diagnostic Approaches for Detecting Metallo-β-Lactamase–Producing Gram-Negative Bacteria: A Review of MALDI-TOF Mass Spectrometry, Culture Methods, and Emerging Machine Learning Applications, Vascular and Endovascular Review, Vol.9, No.1, 351-355

INTRODUCTION

Antimicrobial resistance is recognized as a major public health concern worldwide, with metallo-β-lactamase (MBL)–producing Gram-negative bacteria classified as high-priority pathogens by the World Health Organization [1]. These organisms are capable of hydrolyzing carbapenems and other β-lactam antibiotics, resulting in limited treatment options and increased mortality. The global spread of MBL genes such as blaNDM, blaVIM, and blaIMP among Enterobacterales, Pseudomonas aeruginosa, and Acinetobacter baumannii has been widely reported [2,3].

Conventional culture-based and phenotypic detection methods remain the backbone of laboratory diagnosis; however, their extended turnaround time may delay appropriate clinical management and infection control interventions [4]. Molecular techniques provide accurate gene-level detection but are costly and require specialized infrastructure [8]. In this context, MALDI-TOF MS has significantly improved laboratory workflows by enabling rapid microbial identification and, more recently, functional resistance detection [5]. The integration of machine learning with MALDI-TOF spectral analysis represents an emerging approach aimed at improving diagnostic accuracy and automation. This review provides a critical overview of these diagnostic strategies, with emphasis on their relevance to routine clinical practice.

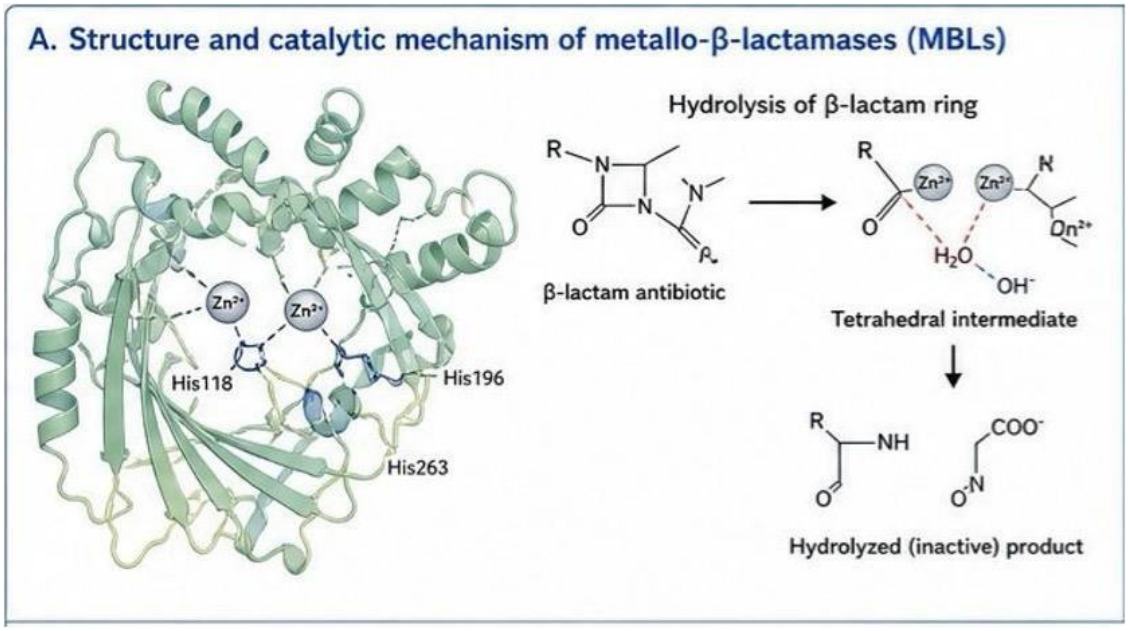


Figure 1. Structure and catalytic mechanism of metallo-β-lactamases (MBLs).

METALLO-β-LACTAMASES AND CONVENTIONAL DETECTION METHODS

Metallo-β-lactamases are zinc-dependent Class B β-lactamases that hydrolyze a wide range of β-lactam antibiotics, including carbapenems [2]. Clinically important MBLs such as NDM, VIM, and IMP are frequently encoded on mobile genetic elements, facilitating rapid dissemination among Gram-negative bacteria [3].

Detection of MBL-producing organisms traditionally involves culture-based antimicrobial susceptibility testing followed by phenotypic confirmatory assays, including the combined disk test, double-disk synergy test, and modified Hodge test [4]. While these methods are inexpensive and widely available, they demonstrate variable sensitivity and specificity and typically require 24–48 hours after bacterial isolation [9]. Molecular assays such as PCR and whole-genome sequencing allow precise identification of resistance genes but do not always correlate with phenotypic expression and remain inaccessible in many resource-limited settings [8].

MALDI-TOF MASS SPECTROMETRY FOR MBL DETECTION

MALDI-TOF MS identifies microorganisms by analyzing protein mass spectral fingerprints, enabling rapid and accurate identification once bacterial colonies are available [5]. Beyond identification, MALDI-TOF MS has been adapted for resistance detection through carbapenem hydrolysis assays that identify antibiotic degradation products [6,7].

Modified MALDI-TOF protocols incorporating metal chelators such as EDTA allow inhibition of zinc-dependent MBL activity, facilitating differentiation from other carbapenemase classes [7]. These assays provide functional detection within a few hours and demonstrate good diagnostic performance. However, lack of standardization instrument variability, and the need for expert spectral interpretation remain important limitations. Further validation and harmonization are required before widespread clinical adoption.[8].

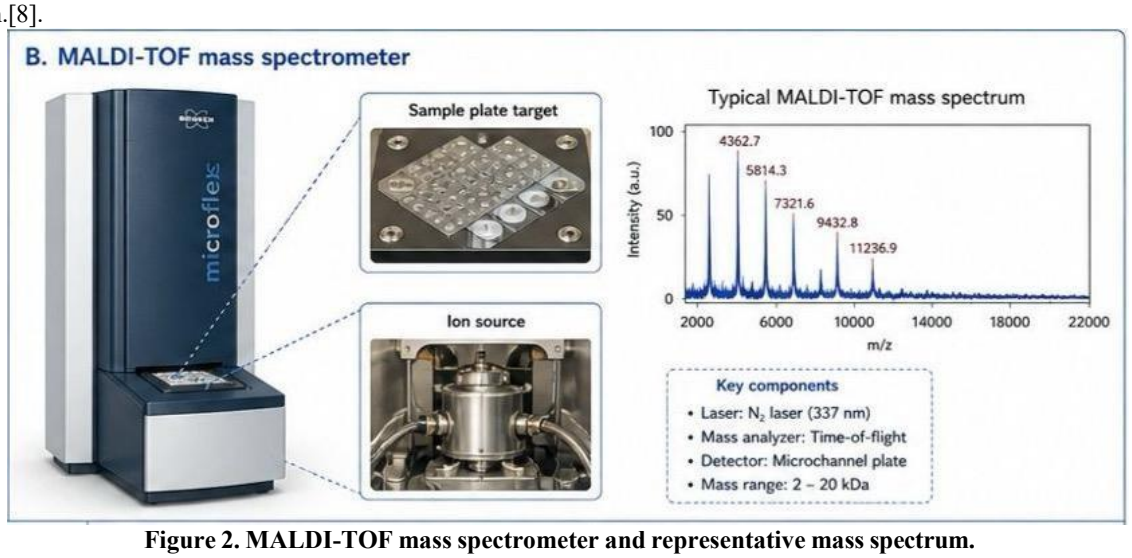
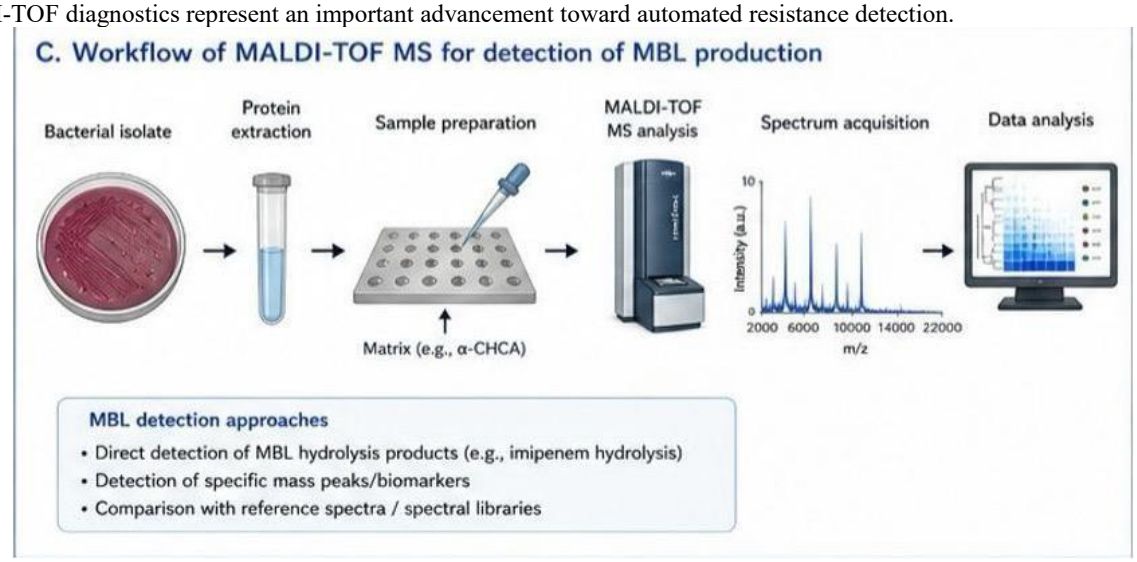


Figure 2. MALDI-TOF mass spectrometer and representative mass spectrum.

MACHINE LEARNING APPROACHES FOR MALDI-TOF–BASED MBL DETECTION

MALDI-TOF spectral data are high-dimensional and complex, making manual interpretation challenging. Machine learning algorithms enable automated analysis by identifying spectral patterns associated with antimicrobial resistance [12]. Typical workflows include spectral preprocessing (baseline correction, normalization, alignment), feature extraction (peak-based or binned spectra), and supervised model training. [13].

Algorithms such as support vector machines, random forests, and neural networks have demonstrated promising accuracy in predicting MBL production directly from MALDI-TOF spectra [13,14]. Deep learning approaches may further enhance performance but require large datasets and careful validation to avoid overfitting [15]. While still under evaluation, ML-assisted MALDI-TOF diagnostics represent an important advancement toward automated resistance detection.



Research Gap and Rationale for the Present Study

Despite substantial progress, several critical gaps remain in the current literature. First, most MALDI-TOF–based carbapenemase detection studies focus on functional hydrolysis assays rather than direct spectral pattern recognition, limiting automation and scalability. Second, machine learning studies often use small, single-center datasets without external validation, raising concerns regarding generalizability. [6,7,8] Third, few investigations specifically target metallo-β-lactamase producers as a distinct diagnostic category, despite their unique biochemical characteristics and clinical importance. Fourth, the lack of standardized preprocessing pipelines and feature extraction strategies hampers reproducibility across laboratories and instruments.

These limitations highlight the need for a comprehensive diagnostic framework that integrates modified MALDI-TOF MS with robust machine learning algorithms, standardized preprocessing, and rigorous validation against molecular reference standards. The present PhD work is designed to address these gaps by systematically comparing conventional phenotypic methods, MALDI-TOF MS, and ML-enhanced MALDI-TOF MS for the detection of MBL-producing Gram-negative bacteria. By combining functional assays, spectral machine learning, and explainable AI, this research aims to establish a clinically deployable, automated diagnostic approach with improved accuracy, speed, and reproducibility.

COMPARATIVE ANALYSIS OF DIAGNOSTIC APPROACHES

MALDI-TOF spectral data are high-dimensional and complex, making manual interpretation challenging. Machine learning algorithms enable automated analysis by identifying spectral patterns associated with antimicrobial resistance. Typical workflows include spectral preprocessing, feature extraction, and supervised model training.

Algorithms such as support vector machines, random forests, gradient boosting models, and neural networks have demonstrated promising accuracy in predicting MBL production directly from MALDI-TOF spectra. Deep learning approaches may further enhance performance but require large datasets and careful validation to avoid overfitting. Model interpretability, data standardization, and external validation remain critical challenges for clinical implementation. [12–14].

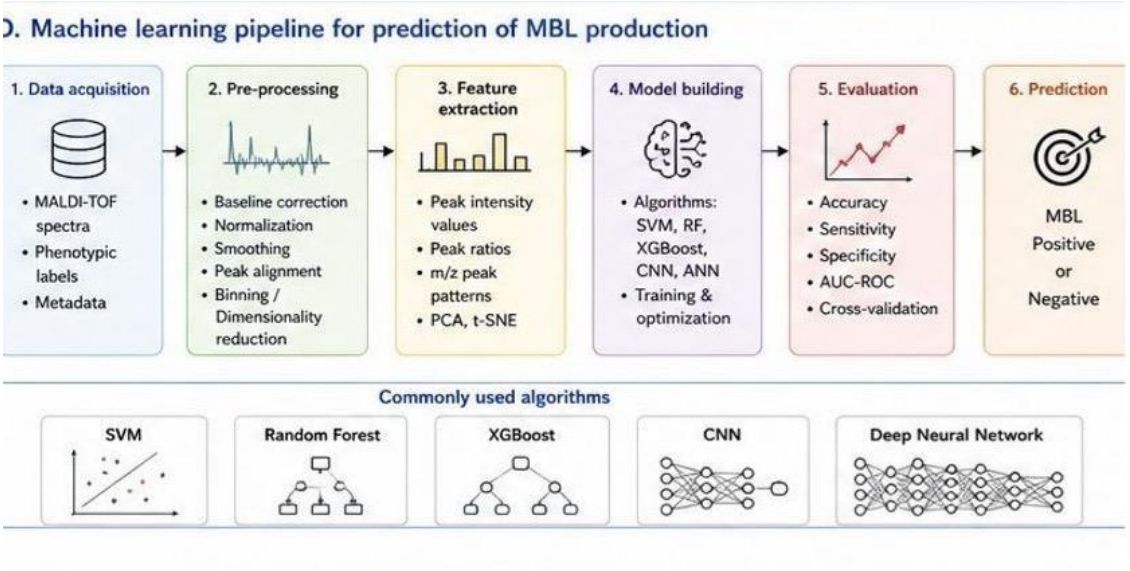


Figure 3. Workflow of MALDI-TOF MS for detection of MBL production.

CHALLENGES AND FUTURE DIRECTIONS

Despite promising results, several challenges hinder routine implementation of MALDI-TOF–based and ML-assisted MBL detection. These include lack of standardized protocols, inter-instrument variability, limited availability of large, annotated datasets, and concerns regarding model interpretability. Regulatory approval, data governance, and integration into laboratory information systems also represent important translational barriers.

Future research should prioritize multi-center validation, harmonization of spectral preprocessing pipelines, and development of explainable AI models to support clinical trust. The incorporation of transfer learning, federated learning, and domain adaptation techniques may further enhance cross-instrument and cross-site generalizability. Integration with real-time clinical decision support tools will be essential for clinical translation.

CONCLUSION

Despite promising results, several challenges hinder routine implementation of MALDI-TOF–based and ML-assisted MBL detection. These include lack of standardized protocols, inter-instrument variability, limited availability of large, annotated datasets, and concerns regarding model interpretability. Future research should focus on multi-center validation, harmonization of spectral preprocessing pipelines, and development of explainable AI models. Integration with laboratory information systems and real-time clinical decision support tools will be essential for clinical translation.

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