**Applications of Mass Spectrometry in Clinical Microbiology**

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# **I. INTRODUCTION**

Mass spectrometry (MS) is a powerful analytical technique used to measure the mass-to-charge ratio of molecules within a sample. It enables the identification and quantification of molecules based on their mass and charge, offering precise and reliable insights into the molecular composition of complex samples [1]. Due to its exceptional analytical capabilities, MS has become an invaluable tool in clinical microbiology. MS encompasses several types and techniques, each designed for specific applications. Electrospray ionization (ESI), direct analysis in real-time (DART-MS), and matrix-assisted laser desorption/ionization (MALDI) are methods used for the ionization of whole proteins [2]. Besides, some specialized types of MS include tandem mass spectrometry (MS/MS) and gas chromatography-mass spectrometry (GC-MS) [3]. This chapter provides an overview of MS and its applications in clinical microbiology, including microbial identification, antimicrobial susceptibility testing (AST), bacterial toxin detection, and environmental and foodborne pathogen analysis. Additionally, this chapter explores the challenges associated with implementing MS in clinical laboratories.

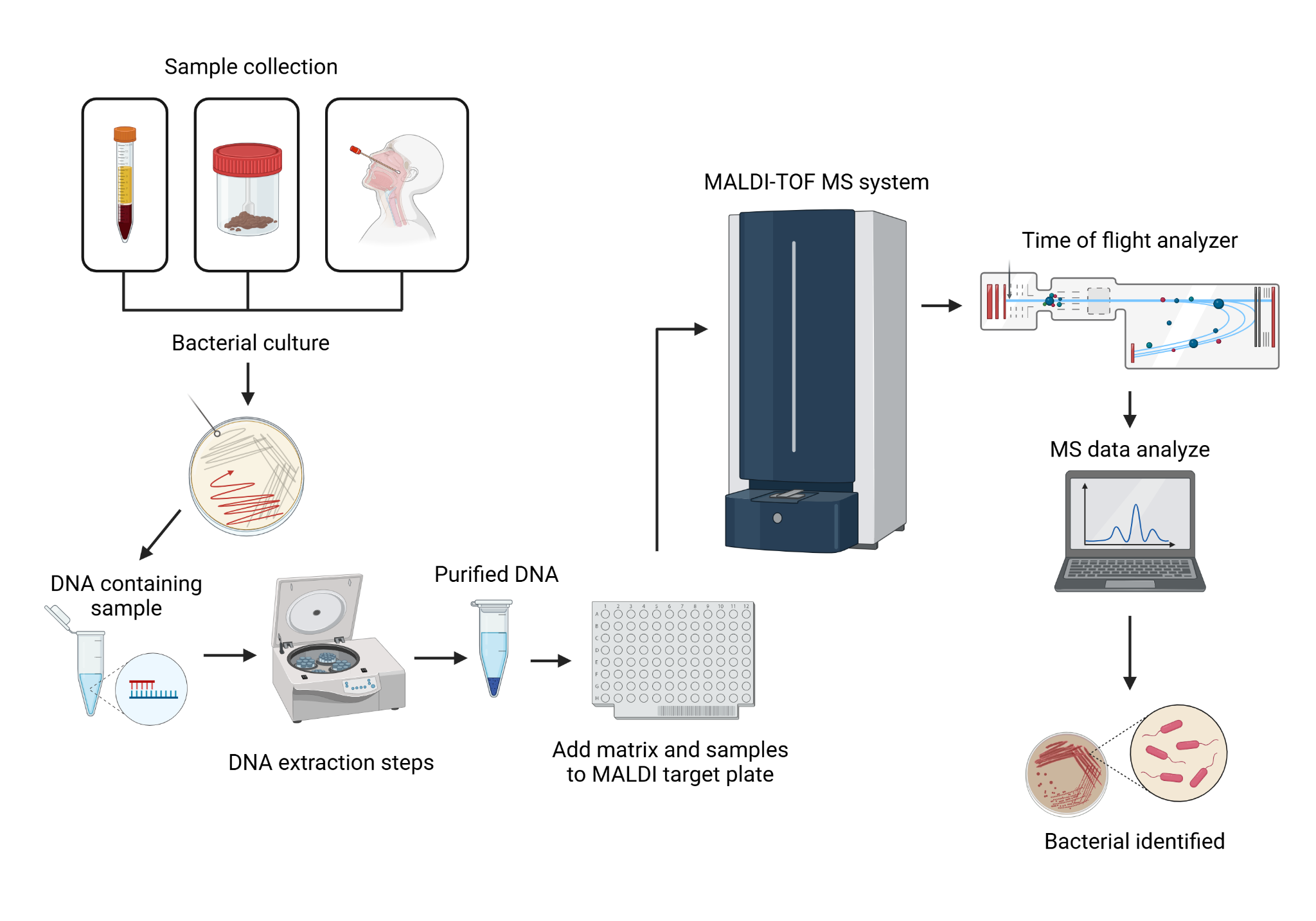
# **II. MICROBIAL IDENTIFICATION**

Microbial identification in clinical diagnostic microbiology laboratories has traditionally relied on biochemical reactions or phenotypic characteristics. While these methods can provide accurate identification, they are often time-consuming, costly, and require the expertise of senior technicians for precise interpretation. The advent of MALDI-TOF MS has transformed the landscape of microbial identification, offering a rapid and efficient approach to detecting and characterizing microorganisms [4]. This technology is capable of sequencing proteins, mapping biomolecules within tissues, and detecting epidemic lineages, making it an invaluable tool in both clinical and research settings.

Unlike conventional methods, which can take hours or days, MALDI-TOF MS can identify microorganisms within minutes by directly analyzing colonies. This reduction in diagnostic time has had a profound impact on clinical workflows, enabling faster initiation of targeted treatments. MALDI-TOF MS relies on pre-established mass spectra databases or automated identification software, such as BioTyper (Bruker Daltonics), SARAMIS (Shimadzu & Anagnostec), and MicrobeLynx™ (Waters Corporation), which integrate proprietary databases for accurate microbial identification [5]. A study comparing the performance of two MALDI-TOF MS systems, the BD Bruker Biotyper, and Vitek MS, demonstrated significant differences in their accuracy when identifying anaerobic isolates. The Bruker Biotyper system outperformed the Vitek MS, achieving higher identification rates at both species (85.3% vs. 65.5%) and genus (89.7% vs. 71.2%) levels while also showing fewer instances of misidentification and non-identification. Despite the effectiveness of MALDI-TOF MS in microbial diagnostics, its success relies on the comprehensiveness of its spectral databases. Expanding these databases by adding more reference spectra for clinically relevant bacteria can further improve the precision of identification. Additionally, continuous database updates are important to ensure that MALDI-TOF MS remains a reliable and essential tool for microbial identification in clinical settings [6].

The principle behind MALDI-TOF MS involves the use of a UV laser to ionize proteins within a microbial sample, with the aid of a matrix that absorbs the laser energy and facilitates desorption and ionization. These ions are analyzed to determine their mass-to-charge ratios, producing a unique protein "fingerprint" that is compared to known spectra in a database for identification [7], [8]. This approach offers significantly faster and more accurate results compared to traditional phenotypic methods, such as polymerase chain reaction (PCR) or genome sequencing.

MALDI-TOF MS uses two main methods for microorganism characterization. The first method relies on fingerprint databases to compare mass spectra. Unique spectra of intact cells are generated and matched with available fingerprint libraries, offering quick results that are easily adapted for routine diagnostic use. The second method matches biomarker masses to a proteome database, identifying unknown microorganisms by comparing biomarker protein masses in the spectrum with predicted molecular masses derived from genomic data. This method accommodates variations in protein profiles, accounting for differences in culture growth rates and sample processing conditions [9].

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Figure 1. MALDI-TOF MS Workflow in Microbial Identification. *The image was regenerated using Biorender Premium 2025***

**II.A. Identification of bacteria at the species level**

MALDI-TOF MS has demonstrated remarkable accuracy in identifying bacteria at the genus and species levels, including both Gram-negative and Gram-positive isolates [10]. By ionizing a sample matrix with a laser beam, MALDI-TOF MS generates a unique mass spectrum that represents the protein profile of the microorganism. This spectrum is matched against a database of known spectra using pattern-matching algorithms. Scores of ≥2.0 indicate reliable species-level identification, while scores between 1.7 and 2.0 suggest genus-level identification [10].

In one study, the variability of 17–25 mass peaks in the mass spectral fingerprints of each strain was used to identify 32 strains from 17 *Aeromonas* species at both the species and strain levels [11]. The results were validated by comparison with databases containing 45 reference strains of 17 *Aeromonas* species to blindly identify 52 *Aeromonas* strains from drinking water samples. MALDI-TOF MS successfully identified 82.7% of the 52 environmental strains, compared to biochemical methods used as a positive control [4].

Further evidence of MALDI-TOF MS's high accuracy was demonstrated in a 2010 study involving bloodstream infection samples. Using the BioTyper database, the technology accurately identified 100% of *Staphylococcus aureus* isolates and 99.1% of coagulase-negative *Staphylococcus* (CoNS) isolates from 450 staphylococcal samples [12]. These findings, validated against PCR sequencing of the rpoB gene, highlight MALDI-TOF MS as a reliable tool for bacterial identification.

Clinical microbiological laboratories increasingly apply this technique. However, its effectiveness has been limited by the availability of spectra databases that primarily cover clinically relevant bacteria, making it less comprehensive for highly pathogenic bacteria (HPB). To address this limitation, a project was initiated to fix the weakness of MALDI-TOF MS in the diagnostics of HPB by developing protocols for reliable microbial inactivation and mass spectra acquisition. As a result, publicly available databases now include spectra for HPB, closely related bacterial strains, and clinically relevant microorganisms. Platforms such as ZENODO provide access to datasets containing 11,055 spectra from 1,601 microbial strains across 264 species, significantly improving the tool’s ability to identify bacterial strains with high accuracy. This advancement enables more precise strain differentiation, aiding in outbreak investigations, epidemiological studies, and the tracking of bacterial evolution. By leveraging these expanded databases, MALDI-TOF MS continues to evolve as an indispensable tool for microbial diagnostics, ensuring more comprehensive identification and improved public health responses [13].

The implementation of MALDI-TOF MS in clinical microbiology has significantly enhanced microbial identification, with Bruker Biotyper, VITEK-MS (bioMérieux), AXIMA MALDI-TOF (Shimadzu), and Zybio EXS 2600 being among the most widely used systems (Table 1). Each system relies on proprietary databases: Bruker Biotyper (Bruker Taxonomy Database, 4,320 species), VITEK-MS (SARAMIS™, 1,316 species), AXIMA MALDI-TOF (SARAMIS™ and SuperSpectra™, 40,000 mass spectra), and Zybio EXS 2600 (4,000 species). Early comparative study demonstrated that both Bruker and Shimadzu systems significantly reduced identification turnaround time and costs compared to traditional biochemical methods, with Bruker achieving 99.1% accuracy and Shimadzu 99.4% accuracy for routine bacterial identification [14]. A more recent head-to-head evaluation by Dichtl et al. (2023) showed that Bruker Biotyper had the highest valid identification rate (98.6%) and 0% misidentification rate, while VITEK-MS demonstrated the highest agreement with 16S rRNA sequencing (99.7%) [15]. AXIMA MALDI-TOF benefits from enhanced spectral reproducibility with SuperSpectra™, and Zybio EXS 2600, though newer, achieved a strong 98.5% agreement with sequencing. While all systems provide high accuracy, the choice depends on clinical needs, species coverage, and database integration, with Bruker excelling in broad-spectrum identification, VITEK-MS in clinical validation, Shimadzu in spectral stability, and Zybio as a rising competitor in microbial diagnostics.

**Table 1. Different MALDI-TOF systems with their associated databases for microbial identification.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **MALDI-TOF SYSTEM** | **Year of Release** | **Associated Database for Identification** | **Number of available Species with Reference Spectra on Database (updated to 2024)** | **References** |
| Vitek-MS (Biomerieux) | 2011 | VITEK® MS Knowledge Base | -Includes spectra from over 15,000 distinct microorganism strains with an average of 12 strains per species.  -1,316 total organisms, with FDA 510(k) clearance for 401 organisms. Among this includes 39 mycobacteria, 16 Nocardia species, and 207 molds and yeasts and other claim/unclaimed organisms. | [16] |
| Bruker Biotyper | 2006 | - MBT Compass reference library (2023)  - MBT IVD reference library (version 2023)  - MALDI Biotyper® CA library | - The MBT Compass reference database includes 4,320 species spanning 712 microbial genera, making it suitable for research laboratories and industrial applications such as food safety, veterinary diagnostics, and pharmaceutical environments.  - The MBT IVD reference database contains spectra from thousands of microbial strains, covering 4,239 species across 686 genera. It includes both commonly encountered clinical pathogens and rare microorganisms, enhancing its utility in clinical microbiology.  -The FDA-cleared MALDI Biotyper® CA System workflow consists of two distinct sections:  +Clinically validated species – The MALDI Biotyper® CA library comprises 488 microbial species, categorized into anaerobes, gram-positive and gram-negative bacteria, and yeasts. These species have undergone clinical validation for diagnostic use.  +Non-clinically validated species – This library contains reference spectra for over 3,400 species that have not been clinically validated. To ensure public health safety, these species are specifically marked in the MALDI Biotyper® result report, prompting laboratories to confirm identification through alternative methods. Additionally, results from non-clinically validated species cannot be transmitted from the MBT CA system to laboratory information systems. | [17] |
| MALDI-TOF AXIMA (Shimadzu Cooperation) | 2007 | SARAMIS™/ SuperSpectra™ (1999—regularly updated until now) | - A comprehensive spectral archive that integrates both SuperSpectra™ and reference spectra derived from well-characterized isolates. These isolates are validated through widely accepted microbiological methods, including phenotyping, 16S rRNA sequencing, and Multi-Locus Sequence Typing**.**  - Each SuperSpectrum is made up of several reference spectra (mass profiles) that were collected from at least eight different isolates of the same species at different times and under different growth conditions.  - The combined SARAMIS™ and SuperSpectra™ database currently holds approximately 40,000 mass spectra, allowing for the identification of both common and rarely detected microbial species, making it a highly valuable tool in clinical microbiology  - Since its initial release in 1999, the SARAMIS™ database has expanded significantly, originally covering 2,000 species and 500 genera but now containing over 2,800 SuperSpectra™ entries for bacterial and fungal identification. | [18] |
| Zybio EXS 2600 | 2020 | v3.0.2.2 (2024) | - Proprietary database containing over 4,000 local microbial species, including bacteria and fungi, with ongoing expansions to enhance identification accuracy | [19] |

## **II.B. Identification of bacteria at the subspecies or strain levels**

While MALDI-TOF MS is highly effective for species-level bacterial identification, achieving strain-level differentiation can be challenging. Advanced techniques, such as specialized reference databases and sophisticated data analysis algorithms, enable the detection of subtle variations in protein profiles to distinguish strains within a species. However, these methods may not always provide reliable strain-level identification in all cases [9]. The strain-level resolution has been achieved for various bacteria using library-based and bioinformatics-driven approaches. This includes three key types of strain-level characterization: strain categorization, differentiation, and identification. Enhancements to library-based methods often involve sample pre-treatment, such as extraction or purification of specific biomolecules (e.g., lipids or proteins), and data reduction strategies to refine spectral quality [20].

Strain-level identification relies on detecting subtle variations in the mass spectra, such as differences in protein expression or minor modifications in ribosomal proteins and other biomolecules. Bioinformatics tools, including machine learning algorithms and multivariate analysis, are used to analyze these fine spectral differences. Techniques like tandem MS or MS/MS can further enhance resolution by identifying unique peptide markers associated with specific strains. Furthermore, integrating genomic data into MALDI-TOF workflows has proven valuable [9]. By correlating specific spectral peaks with genetic differences, researchers can improve the accuracy of strain-level identification. Applications include the identification of antibiotic-resistant strains, tracking outbreak strains in epidemiology, and distinguishing pathogenic strains from non-pathogenic ones.

Integrating genomic data with MALDI-TOF workflows has proven particularly valuable. By correlating specific spectral peaks with genetic differences, researchers can improve the accuracy of strain-level identification. Applications include identifying antibiotic-resistant strains, tracking outbreak strains in epidemiological investigations, and distinguishing pathogenic strains from non-pathogenic ones.

In one notable study, cluster analysis using bioassays identified 16 *Pseudomonas putida* isolates at the strain level. Phylogenetic classification of *P. putida* using MALDI-TOF MS was conducted on 43 putative species, and strain-specific bioions were identified through comparative analysis [21]. These biomarkers were further predicted from MALDI-TOF MS spectra by referencing genome sequences from cell lysates and purified ribosomal proteins of the *P. putida* strain KT2440 [8]. The phylogenetic data obtained from MALDI-TOF MS matched DNA gyrase subunit-B gene sequences, demonstrating consistency between the methods. Moreover, MALDI-TOF MS revealed diverse mass profiles among biovars A and B of *P. putida* strains, which were accurately classified using phylogenetic techniques.

# **III. ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)**

MS can be used for AST by detecting metabolic changes or the degradation of antibiotics in the presence of pathogens. MALDI-TOF-based AST is a rapid and efficient method for determining bacterial antibiotic susceptibility. This technique analyzes the unique protein profiles of bacteria after exposure to different antibiotic concentrations, enabling the quick identification of resistant strains [22]. Compared to traditional methods like broth dilution or disk diffusion, MALDI-TOF-based AST offers significantly faster results. The process starts with exposure to varying antibiotic concentrations. Then, bacterial cells are prepared and analyzed using MALDI-TOF MS. The resulting protein spectra are compared to a reference database to detect changes in protein expression, providing insights into antibiotic resistance mechanisms.

MALDI-TOF MS has been successfully applied to determine antibiotic susceptibility in urinary tract infections (UTIs) directly from patients’ urine samples. This approach enables the rapid identification of pathogens and AST for Gram-negative UTIs within just 4.5 hours of receiving the samples [4]. However, while promising, it is still under development and not widely used in routine clinical practice for all types of bacteria and antibiotic combinations.

## **III.A. Detection of resistant isolates**

MALDI-TOF MS has been applied to pathogen identification by combining multiple techniques to enhance its diagnostic capabilities. These include detecting mass spectral differences between susceptible and resistant isolates of a given microorganism using classical strain-typing methods, analyzing bacterial hydrolysis of β-lactam antibiotics, detecting stable (non-radioactive) isotope-labeled amino acids, and assessing bacterial growth in the presence and absence of antibiotics using an internal standard. These advancements have significantly improved the ability of MALDI-TOF MS to detect drug resistance across a broad range of microorganisms, including viruses.

A diagram of a scientific experiment

AI-generated content may be incorrect.

**Fig 2. The MALDI-TOF MS process detects β-lactamase producers through the hydrolysis of the target β-lactam antibiotic. *The image was regenerated using Biorender Premium 2025***

The success of MALDI-TOF MS in accurately differentiating methicillin-resistant *S. aureus* (MRSA) from methicillin-susceptible *S. aureus* (MSSA) has proven valuable for guiding antibiotic selection and improving diagnostic outcomes. Specific spectral peaks characteristic of MRSA and MSSA have been identified, and variations in the mass spectra of MRSA isolates may allow differentiation between clonal complexes [23]. However, while MALDI-TOF MS can distinguish MRSA isolates, its ability to subtype and classify *S. aureus* at the level of individual clonal complexes remains limited and is still under investigation.

MALDI-TOF MS has also demonstrated the ability to differentiate vancomycin-resistant from vancomycin-susceptible *Enterococcus faecium* by detecting the presence or absence of specific peaks in *vanB*-positive cases. In comparison to PCR, MALDI-TOF showed higher sensitivity and specificity. A 2015 study testing *Enterococcus* strains reported that the method for detecting vancomycin-resistant *enterococci* (VRE) achieved a sensitivity of 80% and a specificity of 90%, highlighting MALDI-TOF MS as an effective clinical tool for combating VRE pathogens [24].

## **III.B. Monitoring of antibiotic efficacy**

MS can be applied to evaluate the effectiveness of different antibiotic concentrations on bacterial strains. A laser desorption/ionization (LDI)-MS-based method has been developed to assess bacterial viability using redox dyes as indicators. Resazurin (RS), a redox dye, absorbs the laser energy and undergoes ionization without requiring the addition of a matrix for MS analysis [24]. Both the peaks of RS and its reduction product, fluorescent resorufin (RF), can be accurately detected by LDI-MS. Bacterial cell viability is then assessed based on the peak ratio of RF to RS. This approach has been used to study changes in the viability of pathogenic bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *S. aureus*, in the presence of antibiotics such as ciprofloxacin, ampicillin, tetracycline, oxytetracycline, and levofloxacin. The LDI-MS-based assay is faster and more sensitive than the optical absorption method, which uses the same redox dye as an indicator [24].

# **IV. DETECTION OF ANTIMICROBIAL RESISTANCE MECHANISMS**

MALDI-TOF MS-based methods have shown great potential in detecting antibiotic resistance. This technology has successfully identified antimicrobial resistance across various classes of antibiotics in numerous clinically significant bacterial species. These include members of the *Enterobacteriaceae* family, non-fermenting Gram-negative bacteria, Gram-positive cocci, anaerobic bacteria, and mycobacteria. These advancements highlight the promise of MALDI-TOF MS in facilitating further clinically important developments in this field [22].

Most research on this topic focuses on three main mechanisms: (1) detecting β-lactamase activity by visualizing the hydrolysis of the β-lactam ring; (2) identifying biomarkers responsible for or associated with drug resistance or non-susceptibility; and (3) analyzing differences in proteomic profiles of bacteria incubated with or without antimicrobial drugs [22]. Early detection of drug resistance is a critical priority for clinicians, as it enables the rationalization of antibiotic therapy and improves patient outcomes. MALDI-TOF MS offers a significant advantage in saving time during the diagnostic process, making it an invaluable tool when rapid and effective antibiotic treatment is essential for patient survival.

## **IV.A. Identification of antibiotic resistance markers**

MALDI-TOF has proven effective in detecting hydrolyzed antibiotics resulting from β-lactamase activity, making it a reliable method for assessing β-lactam susceptibility and resistance [23]. The β-lactam antibiotic group includes various types, all of which share a β-lactam ring in their molecular structure. The primary resistance mechanism against most β-lactam antibiotics involves the destruction of this β-lactam ring through the expression of β-lactamase, rendering the antibiotics ineffective. Many research outcomes revealed the hydrolysis of the β-lactam ring after exposure of β-lactam antibiotics to β-lactamase-producing (aerobic and anaerobic) bacteria could be revealed in mass spectra by a decrease of the peak corresponding to the antibiotic and the appearance of peaks representing its hydrolysis products [22].

The applicability of the MALDI-TOF method has been demonstrated in various Gram-negative bacteria, including *E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Acinetobacter baumannii*, and *Enterobacter cloacae*. Additionally, it has been successfully applied to assess resistance against different β-lactam antibiotics, such as ampicillin, piperacillin, cefotaxime, ceftazidime, ertapenem, imipenem, and meropenem [2]. Another successful application of MALDI-TOF MS is the screening of carbapenemase activity directly from blood culture bottles inoculated with human blood and spiked with *Bacteroides fragilis*. This approach detects carbapenemase production in *Bacteroides fragilis* strains harboring the cfiA gene, which encodes for carbapenemase [25]. The method has been used to differentiate strains based on their ertapenem minimum inhibitory concentrations (MICs).

MALDI-TOF MS has been effectively applied to detect carbapenemases in Gram-negative bacteria. One study utilized commercial antibiotic susceptibility test disks as a source of ertapenem substrate in a MALDI-TOF MS-based assay to identify carbapenemase-producing Enterobacterales (CPE). The assay was validated on 48 CPE isolates from eight different species expressing NDM-, VIM-, KPC-, and OXA-48-type carbapenemases, which exhibited a wide range of carbapenem resistance levels (MIC range: 0.25–>32 mg/L) [26]. Additionally, the assay was tested on 48 carbapenemase non-producing isolates. No hydrolysis peaks were detected for carbapenemase-negative strains, and intact ertapenem peaks remained unchanged, confirming the assay's specificity. Another study used LC-MS/MS assay to detect the proteins responsible for resistance, including KPC, OXA-48-like, NDM, and VIM carbapenemases in *E. coli* and *K. pneumoniae* simultaneously. The method was validated using 83 isolates of *K. pneumoniae* and *E. coli* by defining carbapenemase-specific target peptides through comparative analysis of GenBank sequences. The assay detected all carbapenemases with 100% specificity and no peptide carry-over, demonstrating its accuracy and reliability [27]. This approach offers a direct and precise alternative to traditional phenotypic and molecular techniques for detecting antimicrobial resistance.

Additionally, a study compared MALDI-TOF MS method performance with real-time PCR detection of carbapenemase genes (blaKPC, blaOXA-48, and blaNDM) and determined carbapenem MICs using imipenem, meropenem, and ertapenem. Positive and negative controls were included for validation, using *K. pneumoniae* NCTC 13438 and *E. coli* ATCC 25922, respectively. Out of the 74 isolates, 65 were resistant to carbapenems, and nine were susceptible [28]. PCR analysis identified blaOXA-48 in 57 isolates, blaNDM in 15 isolates, and blaKPC in four isolates, with 11 strains harboring both blaOXA-48 and blaNDM genes. No carbapenemase genes were detected in the nine susceptible strains. The MALDI-TOF MS method demonstrated a sensitivity of 83.1% at the second hour of incubation and reached 100% at the fourth hour, with 100% specificity across both time points and no false-positive results. The improved sensitivity with extended incubation highlights the importance of optimizing incubation time to enhance detection accuracy [29]. That MALDI-TOF MS directly detects carbapenemase activity, providing a more functional assessment of resistance makes practical advantages of MALDI-TOF MS for routine diagnostic microbiology [29].

MS can be used to detect methicillin resistance in bacteria, specifically by identifying unique protein markers associated with resistant strains like MRSA through their distinct mass-to-charge ratio in the mass spectrum; this allows for rapid identification of resistant bacteria compared to traditional culture methods [22]. Detecting penicillin-binding protein 2a (PBP2a) is a common method for identifying MRSA. Initial studies of PBP2a revealed the presence of glycan modifications, which contribute to the complexity of this resistance protein and present challenges for drug resistance detection. To address this, a proof-of-concept tandem MS method was developed, involving the generation of N-terminal PBP2a peptide-like fragments and the detection of unique ions through top-down proteomic analysis. This approach successfully identified two PBP2a variants, PBP2amecA and PBP2amecC, across a panel of MRSA strains with diverse genetic backgrounds. Additionally, PBP2amecA was accurately detected in clinical isolates using a rapid five-minute liquid chromatography separation [30]. These findings demonstrate the potential of direct MS-based detection of resistance markers and highlight the advantages of integrating such approaches into clinical diagnostics.

MALDI-TOF MS-based direct-on-target microdroplet growth assay (DOT-MGA) has been applied to detect methicillin resistance in Gram-positive bacteria. A study analyzed 14 consecutively collected MRSA strains, 14 MSSA strains, and a collection of MRSA challenge strains representing various SCCmec types, mec genes, and spa types [31]. Spectra were acquired and evaluated using MALDI Biotyper software. The test was considered valid if the growth control achieved an identification score of ≥1.7, while susceptible isolates were classified with an identification score of <1.7. The method demonstrated a test validity of 96.4%, along with 100% sensitivity and 100% specificity in detecting methicillin resistance in clinical isolates. Furthermore, all strains in the MRSA challenge collection were successfully identified as methicillin-resistant [31]. This highlights the potential of MALDI-TOF MS combined with DOT-MGA as a reliable and accurate method for detecting methicillin resistance in clinical settings.

## **IV.B. Targeted proteomic and lipidomic analyses to identify resistance-conferring mutations or genes**

MS is used to analyze bacterial proteomes by identifying and quantifying the proteins present in a bacterial cell, allowing researchers to detect proteins specifically associated with antibiotic resistance, essentially acting as biomarkers for identifying resistant bacterial strains by comparing their protein profiles to known susceptible bacteria; this approach is crucial for understanding the mechanisms of resistance and developing new therapeutic strategies [32].

Proteomic studies, which investigate the metabolic and protein composition of microorganisms on a global scale, have facilitated the development of high-throughput proteomic technologies to analyze and quantify proteins [33]. MS-based proteomic approaches, including gel-based and gel-free methods, enable researchers to study bacterial regulatory mechanisms, protein modifications, and interactions, offering valuable insights into antimicrobial resistance mechanisms. Techniques such as 2D gel electrophoresis and its advanced variant, 2D-DIGE, have been used to separate and identify proteins, though their sensitivity is limited for small protein quantities or post-translational modifications. Alternatively, gel-free, label-free proteomics, coupled with MS/MS and liquid chromatography (LC), allows for a broader quantification of proteins with simpler sample preparation and large-scale data accumulation. Quantitative proteomics using MS also supports both relative and absolute protein quantification through labeling techniques like SILAC, iTRAQ, and TMT, which enable the identification of differentially expressed proteins under various conditions [34]. These methods have been instrumental in studying bacterial stress responses, microbial-host interactions, and the mechanisms of resistance, thus advancing our understanding of bacterial pathogenesis and facilitating the development of new diagnostic and therapeutic approaches.

MALDI-TOF MS can identify microbial biomarkers, including protein fragments obtained after trypsin digestion, that are associated with antimicrobial resistance. For instance, MRSA positive for the agr (accessory gene regulator) and harboring the class A mec complex was identified through the detection of the small peptide PSM-mec in whole cells. Similarly, resistance markers such as peptide fragments linked to beta-lactamases (CTX-M1, CMY-2, VIM, and TEM) and aminoglycoside-modifying enzymes were detected in clinical *E. coli* strains using periplasm extraction followed by nano-LC separation. Furthermore, capillary-electrophoresis MS has been used to detect carbapenemases like OXA-48 and KPC in multidrug-resistant Gram-negative bacteria [35]. Notably, MALDI-TOF MS has demonstrated greater sensitivity in detecting beta-lactamase proteins compared to PCR-based detection of their corresponding genes, underscoring its potential as a robust tool in resistance diagnostics.

MS can effectively detect modifications to lipid A, a key component of lipopolysaccharides in bacterial membranes, which can indicate resistance to antimicrobial peptides like colistin, as these modifications often alter the lipid A structure and reduce its affinity for the antibiotic, thus conferring resistance [36]. By isolating lipid A from wild-type and *mcr-1*-expressing strains, researchers can use MALDI-MS to track PEtN addition. This is possible because PEtN has a distinct molecular weight (141 Da), and its addition to lipid A results in a detectable mass difference (Δm/z of 123 in negative-ion mode) due to dehydration between PEtN and the lipid A phosphate moiety. Using a lipid matrix such as norharmane, MS spectra in negative-ion mode allow the identification and estimation of the predicted lipid A structures present in each strain. These structural insights are critical for understanding resistance mechanisms conferred by Mcr-1 and can guide the development of targeted therapies [35].

# **V. DETECTION OF SUBSTANCES**

Beyond its applications in microbial identification and resistance profiling, MS serves as a versatile tool in clinical microbiology for substance identification. By leveraging its high sensitivity and specificity, MS enables the precise detection and quantification of critical substances, including pathogenic toxins and antimicrobial agents. These capabilities are essential for diagnosing toxin-mediated diseases and optimizing therapeutic interventions, showcasing the adaptability of MS in addressing diverse clinical challenges.

## **V.A. Detection of pathogenic toxins**

Among the most clinically significant pathogens, *Clostridioides difficile* is a Gram-positive, spore-forming anaerobe responsible for healthcare-associated diarrhea. Its pathogenicity is primarily attributed to two exotoxins, TcdA and TcdB, which damage the intestinal epithelium and trigger inflammation. Additionally, some hypervirulent strains produce a binary toxin, CDT, that may enhance the production of TcdA and TcdB [37]. Rapid and precise detection of these toxins is crucial for diagnosing and managing C. difficile infections. A prominent example MS in detecting pathogenic toxins is its application in identifying *Clostridioides difficile* toxins, specifically Toxin A (TcdA) and Toxin B (TcdB). These exotoxins are pivotal in the pathogenesis of *C. difficile*-associated diarrhea (CDAD), a leading cause of healthcare-associated infections. Traditional diagnostic methods, such as enzyme immunoassays (EIAs), often suffer from suboptimal sensitivity [38]. MS also offers superior multiplexing capabilities, enabling the simultaneous detection of multiple toxins, such as *Clostridioides difficile* TcdA and TcdB, in a single assay, which is not feasible with traditional EIAs. Additionally, MS benefits from integration with extensive spectral databases, allowing automated and accurate identification of toxins, even for rare or novel variants, where EIAs may lack specific antibodies.

MS, particularly MALDI-TOF MS, has been increasingly explored for its ability to differentiate *Clostridioides difficile* toxin-producing strains from non-producers. A recent study developed a MALDI-TOF MS-based detection model, integrating ClinProTools software to enhance the classification of C. difficile isolates recovered from stool samples [39]. Instead of direct toxin detection, bacterial isolates were first cultured and subjected to ethanol-formic acid protein extraction, followed by MALDI-TOF MS spectral acquisition within a mass range of 2,000–20,000 m/z. The resulting spectral patterns were analyzed using ClinProTools, a post-processing software designed for biomarker discovery and microbial classification. To construct a robust detection system, the authors developed classification models using three distinct machine-learning algorithms within ClinProTools: Genetic Algorithm (GA), Supervised Neural Network, and QuickClassifier. These algorithms were trained on 37 toxin-positive and 24 toxin-negative isolates, as well as validated using independent reference strains. The GA model, which selects peak combinations most relevant for strain differentiation, demonstrated high sensitivity (91.7%) when applied to isolates grown on Brucella with hemin and vitamin K agar, though specificity remained moderate. By contrast, when the same model was tested on isolates grown on cycloserine-cefoxitin mannitol agar, specificity improved to 100%, but sensitivity decreased significantly. These findings highlight the critical influence of culture conditions on spectral reproducibility and model performance. By leveraging machine-learning-driven classification, this approach demonstrates the potential of MALDI-TOF MS as a cost-effective, rapid diagnostic tool, aiding clinical microbiologists in distinguishing toxigenic C. difficile from non-producers. Further refinements in database expansion and culture standardization could enhance the robustness of this method for routine clinical application.

The application of MS for *C. difficile* toxin detection directly impacts patient care by facilitating early and accurate diagnosis. Unlike EIAs, which often yield false negatives due to low toxin concentrations, MS demonstrates enhanced sensitivity. For instance, LC-MS/MS has been used to detect pathogenic toxins in stool samples with a limit of detection as low as 1 ng/mL, ensuring accurate identification even in subclinical cases [40]. Additionally, quantitative data provided by MS can guide clinicians in assessing disease severity. Elevated toxin levels correlate with more severe disease phenotypes, aiding in prognostic evaluations and therapeutic decision-making. For example, patients with high TcdA or TcdB levels may benefit from more aggressive treatments, such as fecal microbiota transplantation (FMT) or adjunctive therapies. In outbreak scenarios, MS-based toxin detection facilitates rapid differentiation of toxigenic strains from non-toxigenic *C. difficile* isolates, streamlining infection control measures. Coupled with strain typing, such as whole-genome sequencing, MS enhances the resolution of epidemiological investigations, enabling precise source tracking and containment [41]. Integrating MS with automated sample preparation systems and advanced data analysis algorithms is expected to further improve the accessibility and throughput of toxin detection in clinical laboratories. Advances in high-resolution mass analyzers may also enable the simultaneous detection of multiple toxin isoforms, enhancing diagnostic comprehensiveness.

MS techniques, particularly MALDI-TOF MS, are not solely relevant to *C. difficile* but have also shown effectiveness in identifying other pathogenic toxins, such as Panton-Valentine Leukocidin (PVL) in *Staphylococcus aureus* strains and *Bacillus thuringiensis* toxin.

MALDI-TOF MS is utilized in preliminary research to develop a consistent model for the identification of PVL-producing *S. aureus*. Eighty-one *S. aureus* strains were examined for the presence of Panton-Valentine leukocidin (PVL) and toxic shock syndrome toxin-1 (TSST-1) [42]. The research discovered a peak at a mass-to-charge ratio (m/z) of 4448 as the greatest distinguishing characteristic differentiating PVL-positive from PVL-negative bacteria. A classification model utilizing this peak exhibited a recognition capability of 100% and an overall cross-validation accuracy of 77.07%. Moreover, the potential implementation of this approach facilitated the identification of PVL-producing strains within minutes during clinical care, prior to the results obtained using PCR. This served as a substantial proof of concept for the incorporation of MALDI-TOF MS in expedited toxin detection, highlighting its potential as a point-of-care diagnostic instrument [42].

Furthermore, a prior study conducted in 2002 illustrated the utilization of MALDI-TOF MS for the identification of *Bacillus thuringiensis* (Bt) crystal (Cry) toxins. The work utilized both established Cry toxins (e.g., Cry1Ac and Cry2A) and newly anticipated toxins as controls, confirming that peptide masses derived from in-gel trypsin digestion corresponded precisely to their respective proteins. MALDI-TOF MS effectively distinguished many Cry toxins with analogous molecular weights and isoelectric points from a singular protein band. This approach also identified novel Cry toxins in Bt strains that PCR could not detect the relevant genes for. These findings underscore the efficacy of MALDI-TOF MS as a robust instrument for the identification of Cry toxins, especially in novel Bt strains [43].

In a similar manner, Shiga toxins (Stx) produced by *Escherichia coli* can also be detected effectively using MS, further showcasing its versatility in clinical microbiology. Shiga toxin-producing *E. coli* (STEC) has been increasingly linked to major outbreaks of foodborne illness, with *E. coli* O157:H7 (e.g., strain EDL933) being one of the most studied strains due to its dual possession of the *stx1* and *stx2* genes, along with additional virulence factors. These toxins, Stx1 and Stx2, play a central role in STEC pathogenicity, and their rapid detection is critical for managing public health risks and improving patient outcomes.

Early work demonstrated the feasibility of using MS, coupled with immunoprecipitation, to identify Shiga toxins (Stx1 and Stx2) from STEC. This approach reduced the detection time from two weeks to just two days, providing molecular weight data that confirmed the presence of specific toxins in STEC isolates [44].

Building on these foundations, the application of MALDI-TOF MS is advanced by integrating top-down proteomic techniques to identify Stx2 subtypes (e.g., a, c, d, f, and g) directly from bacterial cell lysates. MALDI-TOF MS was revealed as a rapid and highly specific tool for distinguishing toxin variants based on their mass and sequence-specific fragment ions. Such subtype differentiation is critical for understanding the virulence potential of STEC strains and informing public health interventions during outbreaks [45]. More recently, the scope of MALDI-TOF MS has been expanded by leveraging chemical reduction to enhance the detection of *E. coli* toxin biomarkers, including the B-subunit of Stx [46]. Not only ability of MALDI-TOF MS to identify Shiga toxins was affirmed but the capacity to detect other virulence-associated proteins, such as acid-stress and bacteriophage tail proteins was demonstrated [46].

In conclusion, MS, especially MALDI-TOF MS, has revolutionized pathogenic toxin identification in clinical microbiology with its accuracy, speed, and variety. MS has exceeded EIAs and PCR in sensitivity, specificity, and multiplexing for *Clostridioides difficile* toxins, Shiga toxins, and other virulence-associated proteins. Patient treatment and public health management benefit from its ability to identify various toxin types, distinguish subtypes, and provide quantitative insights. MS also aids epidemic investigations by tracking and containing toxigenic isolates. MS will become even more important to clinical diagnostics as automated sample preparation, high-resolution analyzers, and real-time data processing improve our ability to fight toxin-mediated diseases and protect world health.

## **V.B. Quantification of antimicrobials**

In clinical practice, the standardization of antimicrobial dosing often fails to account for individual patient variability, leading to suboptimal therapeutic outcomes. Factors such as organ dysfunction, critical illness, and genetic differences can significantly alter drug pharmacokinetics and pharmacodynamics, resulting in either subtherapeutic exposure or toxicity. This variability underscores the necessity for precise therapeutic drug monitoring (TDM) to tailor antimicrobial therapy to individual patient needs. TDM involves the measurement of drug concentrations in biological fluids, allowing clinicians to adjust dosing regimens to achieve optimal therapeutic levels while minimizing adverse effects. Incorporating TDM into clinical practice is essential for enhancing patient outcomes and combating the rise of antimicrobial resistance [47].

The application of analytical methods in TDM hinges on their ability to deliver precise, accurate, and reliable measurements of drug concentrations in biological matrices. Among the available methodologies, MS has emerged as a superior tool, offering capabilities that make it exceptionally well-suited for the clinical demands of TDM. Traditional methods, such as immunoassays and high-performance liquid chromatography (HPLC), while effective for routine monitoring, face inherent limitations in specificity, sensitivity, and multiplexing capabilities, which MS overcomes with unparalleled efficiency (Table 2).

**Table 2: Comparison between Mass Spectrometry and conventional methods in Therapeutic Drug Monitoring**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Criteria** | **Immunoassays** | **HPLC** | **Microbiological Assays** | **Mass Spectrometry** | **References** |
| Specificity | Moderate | High | Low | Very High | [48]; [49]; [50]; [51] |
| Sensitivity | Moderate | Moderate | Low | Very High | [48]; [49]; [50] |
| Multiplexing Capability | Limited | Limited | None | Excellent | [48]; [49]; [50]; [51] |
| Time to Result | Rapid | Moderate | Long | Rapid | [49]; [50] |
| Cost | Low | Moderate | Low | High | [48]; [49] |
| Skill Required | Low | Moderate | Low | High | [48]; [49]; [50] |

Unlike immunoassays, which are susceptible to cross-reactivity, or HPLC, which often requires extensive sample preparation, MS can precisely distinguish molecules based on their unique mass-to-charge ratios. This ensures accurate drug quantification in complex biological matrices, such as plasma or cerebrospinal fluid, without interference from metabolites or co-administered agents. Its sensitivity further enables the reliable detection of low drug concentrations, critical for antimicrobials with narrow therapeutic windows like vancomycin and aminoglycosides.

Moreover, MS excels in multiplexing capability, enabling the simultaneous detection and quantification of multiple drugs in a single run. This is a significant advantage over immunoassays and HPLC, which are typically limited to one or a few analytes per assay. For example, LC-MS/MS assays can measure several antimicrobial agents, such as vancomycin, azoles, and aminoglycosides, in a single analysis, streamlining workflows and reducing turnaround time [52]. While immunoassays offer rapid results, their limited multiplexing and accuracy can compromise therapeutic decisions. Microbiological assays, though cost-effective, are impractical in clinical settings due to their low specificity, sensitivity, and long time-to-result.

In terms of accessibility, MS requires a higher level of technical expertise and incurs significant upfront costs compared to the low-cost and user-friendly immunoassays. However, the investment is offset by its superior diagnostic performance, particularly in complex cases where precise drug monitoring is critical. For instance, MS has been instrumental in reducing toxicity risks, such as nephrotoxicity from vancomycin or hepatotoxicity from azole antifungals, by maintaining therapeutic levels within a narrow window [53]. These clinical advantages position MS as an indispensable tool for advancing personalized medicine in TDM, surpassing the limitations of traditional methods while delivering higher-quality patient care.

The MS detection process involves the separation of drug molecules from biological matrices using liquid chromatography, followed by ionization and mass analysis in a tandem mass spectrometer. LC-MS/MS excels in sensitivity by employing multiple reaction monitoring (MRM) to selectively detect drug-specific ion transitions, minimizing interference from other compounds. Internal standards, often isotope-labeled analogs of the target drugs, are used to enhance accuracy and reproducibility. This precision ensures that even low concentrations of antimicrobials, often required for individualized dosing regimens, are reliably quantified.

The application of MS in TDM has evolved significantly over the past two decades. Initially, MS required extensive sample preparation and highly trained personnel, limiting its use to specialized research laboratories. However, advancements in instrumentation and automation have streamlined the workflow, enabling the direct analysis of patient samples with minimal preparation (Table 3).

**Table 3: Advancements in Mass Spectrometry for Antibiotic Quantification in Therapeutic Drug Monitoring**

|  |  |  |  |
| --- | --- | --- | --- |
| **Year** | **Focus/Development** | **Key Findings** | **References** |
| 2020 | - Development of a rapid and simple LC-MS/MS platform for the quantification of 14 antibiotics and a beta-lactamase inhibitor in plasma samples, aimed at enabling therapeutic drug monitoring (TDM) in critically ill pediatric patients. | - Demonstrated excellent accuracy (85.3%–112.7%) and reproducibility (1.3%–9.7%), with minimal matrix effects (<15%) and a recovery rate exceeding 85% for all drugs tested. The assay, validated according to EMA guidelines, featured a turnaround time of 20 minutes and required only 50 µL of plasma, making it suitable for real-time, TDM-guided antimicrobial therapy personalization in clinical settings. | [54] |
| 2021 | - Development and validation of an LC-MS/MS method for quantifying 15 antibiotics in critical care settings. | - Established a fast, sensitive, and quantitative UPLC-MS/MS assay for routine TDM, requiring minimal plasma volume and achieving high precision. | [55] |
| 2022 | Development of an LC-MS/MS method for simultaneous quantification of 18 antibacterial drugs in human plasma for TDM. | - Validated a highly sensitive and precise LC-MS/MS method, successfully applied in routine TDM for 231 clinical samples. | [52] |
| 2022 | Development of a UPLC-MS/MS method for simultaneous quantification of 14 antibiotics in plasma and cerebrospinal fluid. | - Established a rapid and precise method for antibiotic quantification in TDM, successfully applied to 113 patients. | [56] |
| 2023 | Evaluation of the CLAM-2000 automated LC-MS/MS system for 24/7 TDM of beta-lactam antibiotics in routine clinical diagnostics. | - Demonstrated the feasibility of real-time, continuous TDM of beta-lactams using an automated system, ensuring reproducibility and efficiency. | [57] |

Early applications of LC-MS/MS in clinical chemistry laid the groundwork for its routine use in TDM, showcasing exceptional accuracy, reproducibility, and sensitivity for a broad spectrum of antibiotics. For example, a 2020 study introduced a validated LC-MS/MS platform capable of quantifying 14 antibiotics and a beta-lactamase inhibitor in plasma samples with minimal matrix effects and recovery rates exceeding 85%, achieving a rapid turnaround time of 20 minutes. This breakthrough highlighted the potential of LC-MS/MS for real-time, TDM-guided antimicrobial therapy personalization, particularly in critically ill pediatric patients. Such advancements have significantly reduced the complexity of instrumentation and method development, enabling the transition of MS-based antibiotic quantification from specialized laboratories to routine clinical practice [54].

One of the key milestones in this progression was the development of ultra-performance liquid chromatography-tandem MS (UPLC-MS/MS), which introduced significant improvements in analytical throughput and sensitivity. The validation of a UPLC-MS/MS method for quantifying 15 commonly used antibiotics in critical care units provided an efficient approach for routine TDM with minimal sample volume requirements, ensuring rapid turnaround times suitable for clinical decision-making [55]. By enabling the simultaneous quantification of multiple antibiotics, including beta-lactams, linezolid, fluoroquinolones, and daptomycin, this method addressed the high interindividual pharmacokinetic variability observed in critically ill patients, optimizing antimicrobial therapy while reducing the risk of under- or overdosing. Further advancements have expanded the capabilities of LC-MS/MS to include a broader range of antimicrobial agents, ensuring more comprehensive drug monitoring strategies. The development of an analytical platform capable of simultaneously quantifying 18 antibacterial agents in human plasma reinforced the role of MS in personalized medicine, providing clinicians with pharmacokinetic insights essential for dose optimization [52]. The inclusion of antibiotics such as meropenem, cefepime, and levofloxacin, alongside macrolides and glycopeptides, emphasized the necessity of multiplexing in TDM, particularly for patients receiving combination therapies. With rigorous validation ensuring high accuracy, precision, and matrix effect stability, this method has been successfully integrated into routine clinical workflows, further demonstrating MS’s superiority over conventional analytical techniques.

Beyond quantification in plasma, the application of MS for antibiotic detection has expanded to include cerebrospinal fluid (CSF), addressing the critical need for optimized antimicrobial dosing in central nervous system infections. The implementation of a UPLC-MS/MS method for the simultaneous determination of 14 antibiotics in both plasma and CSF has facilitated more precise drug monitoring for infections such as bacterial meningitis and intracranial abscesses [56]. The ability to measure antimicrobial concentrations directly in CSF offers significant clinical advantages by enabling real-time assessment of drug penetration across the blood-brain barrier, guiding clinicians in optimizing dosing regimens to improve therapeutic outcomes while minimizing neurotoxicity risks. The integration of automation in MS-based TDM has further revolutionized clinical workflows, enhancing efficiency and accessibility. The development of an automated sample preparation module, such as the CLAM-2000 system coupled with LC-MS/MS, has demonstrated the feasibility of 24/7 real-time TDM for beta-lactam antibiotics [57]. This advancement addresses the growing need for continuous antibiotic monitoring, particularly in critically ill patients requiring dynamic dose adjustments. By ensuring reproducibility, stability, and minimal manual intervention, automated MS systems contribute to improved patient management by facilitating timely therapeutic interventions and reducing delays associated with traditional batch-based analytical techniques.

Not only is MS widely utilized for antibiotic quantification in therapeutic drug monitoring (TDM), but it has also become an indispensable tool for detecting antifungal drug levels in patients. Antifungal TDM is particularly crucial due to the significant pharmacokinetic variability observed among individuals, the narrow therapeutic index of many antifungal agents, and the need to optimize dosing to maximize efficacy while minimizing toxicity. Among the most frequently monitored antifungal agents are voriconazole, itraconazole, and posaconazole, all belonging to the triazole class. Voriconazole, a first-line agent for invasive aspergillosis and other life-threatening fungal infections, requires careful monitoring due to its nonlinear pharmacokinetics and dose-dependent toxicity. Itraconazole is commonly prescribed for endemic mycoses and prophylaxis in immunocompromised patients, yet its variable absorption necessitates TDM to ensure adequate plasma concentrations. Posaconazole, widely used for prophylaxis against invasive fungal infections, exhibits highly variable bioavailability, which can be significantly influenced by food intake and gastrointestinal function. Given the complexity of antifungal pharmacokinetics, MS has emerged as a highly reliable analytical tool, enabling precise quantification of these drugs in clinical settings. Over the years, the advancement of MS in antifungal detection has been particularly evident in the progressive reduction of analytical run times, allowing for faster and more efficient therapeutic monitoring (Table 4).

**Table 4: Notable advancement in the analytical runtime for antifungal detection using MS**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Published Year** | **Target substance** | **Type of MS** | **Analytical Runtime** | **References** |
| 2010 | Itraconazole, Posaconazole, Voriconazole | HPLC-MS/MS | 13 mins | [58] |
| 2010 | Anidulafungin, Caspofungin, Isavuconazole, Micafungin, Posaconazole, Voriconazole | HPLC-MS/MS | 8 mins | [59] |
| 2010 | Anidulafungin, Caspofungin, Fluconazole, Hydroxyitraconazole , Itraconazole, Posaconazole, Voriconazole, Voriconazole-N-oxide | UPLC-MS/MS | 7 mins | [60] |
| 2017 | Fluconazole, Itraconazole, Posaconazole, Voriconazole | HPLC-MS/MS | 3 mins | [61] |

The application of MS in TDM for antifungal agents has undergone significant advancements, particularly in reducing analytical runtime and enhancing multiplexing capabilities. These developments have positioned MS as a cornerstone technology in clinical practice, enabling precise and efficient quantification of antifungals critical for optimizing therapy and preventing toxicity. Over the years, studies have demonstrated the ability of MS to measure an increasing number of antifungal agents in a single run while significantly decreasing runtime, showcasing its evolution into a highly efficient tool for routine clinical applications.

The year 2010 marked a significant milestone in antifungal TDM, with multiple studies illustrating the utility of MS for this purpose. Baietto et al. developed and validated a HPLC-MS method for the simultaneous quantification of itraconazole, posaconazole, and voriconazole in human plasma. This method, with an analytical runtime of 13 minutes, demonstrated high accuracy and recovery rates, meeting FDA guidelines and ISO standards. Its successful application in clinical settings and international interlaboratory quality control programs underscored its reliability for routine TDM and pharmacokinetic studies [58]. The results represented an important step toward incorporating MS into clinical antifungal monitoring [58]. Later that year, Farowski et al. expanded on this progress by introducing a LC-MS/MS method capable of simultaneously quantifying six antifungal agents across various biological compartments, including plasma, erythrocytes, and leukocytes. The antifungals included triazoles, such as posaconazole and voriconazole, and echinocandins, such as caspofungin and anidulafungin. The method achieved a significantly shorter runtime of 8 minutes while maintaining high precision and sensitivity. By enabling intracellular quantification of antifungal concentrations, the study addressed an unmet clinical need and further demonstrated the capability of MS to streamline antifungal TDM in diverse contexts [59]. Another pivotal study in 2010 was conducted by Decosterd et al., who developed a multiplex UPLC-MS/MS method for simultaneous quantification of eight antifungal agents, including fluconazole, hydroxyitraconazole, and voriconazole-N-oxide [60]. This method required only 100 μL of plasma and achieved a runtime of just 7 minutes, representing a significant improvement in both efficiency and throughput. The method’s high accuracy, minimal matrix effects, and robust performance over clinical concentration ranges demonstrated its suitability for daily routine TDM and clinical research applications, including combination antifungal therapies. The achievements showcased the potential of MS to handle complex pharmacokinetic scenarios in real-time, maximizing both efficacy and safety in antifungal therapy [60].

In 2017, Xiao et al. further advanced the field by developing an HPLC-MS/MS method with an unprecedented analytical runtime of just 3 minutes. This method simultaneously quantified four triazole antifungals—voriconazole, posaconazole, fluconazole, and itraconazole—in human serum. The study highlighted the method’s high precision, accuracy, and excellent correlation with reference laboratories, emphasizing its applicability for routine TDM in clinical settings. The rapid runtime and minimal sample preparation requirements demonstrated the efficiency and practicality of MS for high-throughput clinical workflows, addressing the increasing demand for timely therapeutic monitoring in antifungal management [61].

The application of MS in detecting antimicrobials has revolutionized TDM, offering unparalleled precision, sensitivity, and versatility. As demonstrated in the detection of both antibiotics and antifungals, MS addresses the limitations of traditional methods such as immunoassays and HPLC by enabling simultaneous quantification of multiple drugs, even at trace concentrations, within a single, rapid analytical run. Among the various MS platforms, LC-MS/MS has emerged as the most widely used technique, owing to its exceptional multiplexing capabilities, robustness, and compatibility with clinical workflows. These advancements have solidified MS as an indispensable tool not only in research settings but also in routine clinical practice, where it plays a critical role in optimizing antimicrobial therapy, minimizing toxicity, and mitigating the emergence of resistance. By bridging the gap between laboratory innovation and clinical application, MS continues to set the standard for personalized medicine and antimicrobial stewardship, reinforcing its significance in modern healthcare.

# **VI. MASS SPECTROMETRY USED IN OTHER TYPES OF DETECTION**

## **VI.A. Fungal detection**

MS is considered a highly effective method for identifying clinical fungi, offering a rapid and accurate way to identify various species of yeasts and filamentous fungi, especially when compared to traditional culture-based methods, which can be time-consuming and require specialized expertise, making it a valuable tool in clinical microbiology laboratories [62].

Studies have demonstrated its effectiveness in identifying a wide range of filamentous fungi, such as *Scedosporium* and *Aspergillus*, with technologies like the VITEK MS system achieving an 89% success rate for fungal isolates. The accuracy of identification can be further improved by employing more complex sample preparation methods, which have been shown to achieve species-level accuracy of up to 86.4%. Moreover, expanding databases and integrating in-house libraries have significantly enhanced identification rates, particularly for less common fungal species, where improvements from 0% to 100% were observed for certain *Scedosporium* species [63]. Beyond clinical applications, MALDI-TOF MS has proven effective in environmental monitoring, accurately identifying 92% of fungal isolates in air and dust samples, outperforming traditional microscopy methods. Additionally, innovative approaches like integrating deep learning algorithms with MALDI-TOF MS have demonstrated high accuracy in detecting clonal fungal populations, such as *Aspergillus flavus*. These advancements underscore the versatility and growing significance of MS in fungal detection and identification [64].

MALDI-TOF MS has been successfully applied to identify members of fungal genera such as *Aspergillus, Fusarium, Penicillium, Trichoderma*, and various yeast species like *Candida albicans* from clinical samples. Notably, it excels at discriminating closely related species within the *Candida* genus, such as members of the *C. glabrata* clade (*C. glabrata, C. nivariensis, C. bracarensis*), the "psilosis" complex (*C. parapsilosis, C. metapsilosis, C. orthopsilosis, and Lodderomyces elongisporus*), as well as between *C. albicans* and *C. dubliniensis* [65]. Two primary approaches are employed: intact cell/spore analysis or the extraction of surface proteins for MS measurement. Fungal identification can also be achieved directly from infected plant organs contaminated with fungal spores. The technique relies on peptide and protein profiles within the m/z range of 1000–20,000, where unique biomarker ions facilitate differentiation at the genus, species, or strain level. Accurate identification is enabled through the use of specialized processing software and spectral databases of reference strains, which are most effective when constructed under standardized experimental conditions [66]. Despite the lack of a universal method for fungi, MALDI-TOF MS continues to demonstrate its versatility and potential in both clinical and environmental fungal identification.

The integration of comprehensive database entries has also enhanced the identification of *Cryptococcus neoformans* and *C. gattii*, overcoming early limitations in accuracy. Moreover, MALDI-TOF MS proves to be cost-effective, with lower operating expenses and reduced hands-on processing time compared to conventional and molecular methods. In a study analyzing 303 clinical isolates, MALDI-TOF MS showed high analytical performance, even resolving discrepancies with genotyping techniques like 25S-28S rRNA sequencing [67]. These findings underscore its utility as a reliable, efficient, and cost-effective method for yeast identification in clinical and research settings.

## **VI.B. Viral diagnostics**

MS can detect viral proteins or nucleic acids by analyzing their unique molecular weight and fragmentation patterns, allowing for the identification and quantification of specific viruses within a sample, offering a powerful tool for viral diagnostics and research, particularly when combined with appropriate sample preparation techniques and specialized databases.

MS offers an untargeted approach for virus identification that complements traditional methods like PCR and immunoassays. The viral proteomics workflow (vPro-MS) leverages an extensive peptide library derived from the human genome and a specialized scoring algorithm (vProID score) to accurately identify viral proteins in patient samples. This high-throughput approach, utilizing diaPASEF-based data acquisition, can analyze up to 60 samples per day with a specificity exceeding 99.9%, as demonstrated across 18 different viruses, including SARS, MERS, Ebola, and monkeypox. In the case of SARS-CoV-2, vPro-MS achieves a detection sensitivity comparable to PCR at a cycle threshold of 27, offering quantitative accuracy similar to metagenomic sequencing [68]. Notably, this method facilitates the large-scale screening of biofluids like human plasma, allowing for the discovery of previously undetected viral infections. Researchers and clinicians can enhance pathogen surveillance and improve outbreak response strategies by integrating MS-based proteomics into viral diagnostics.

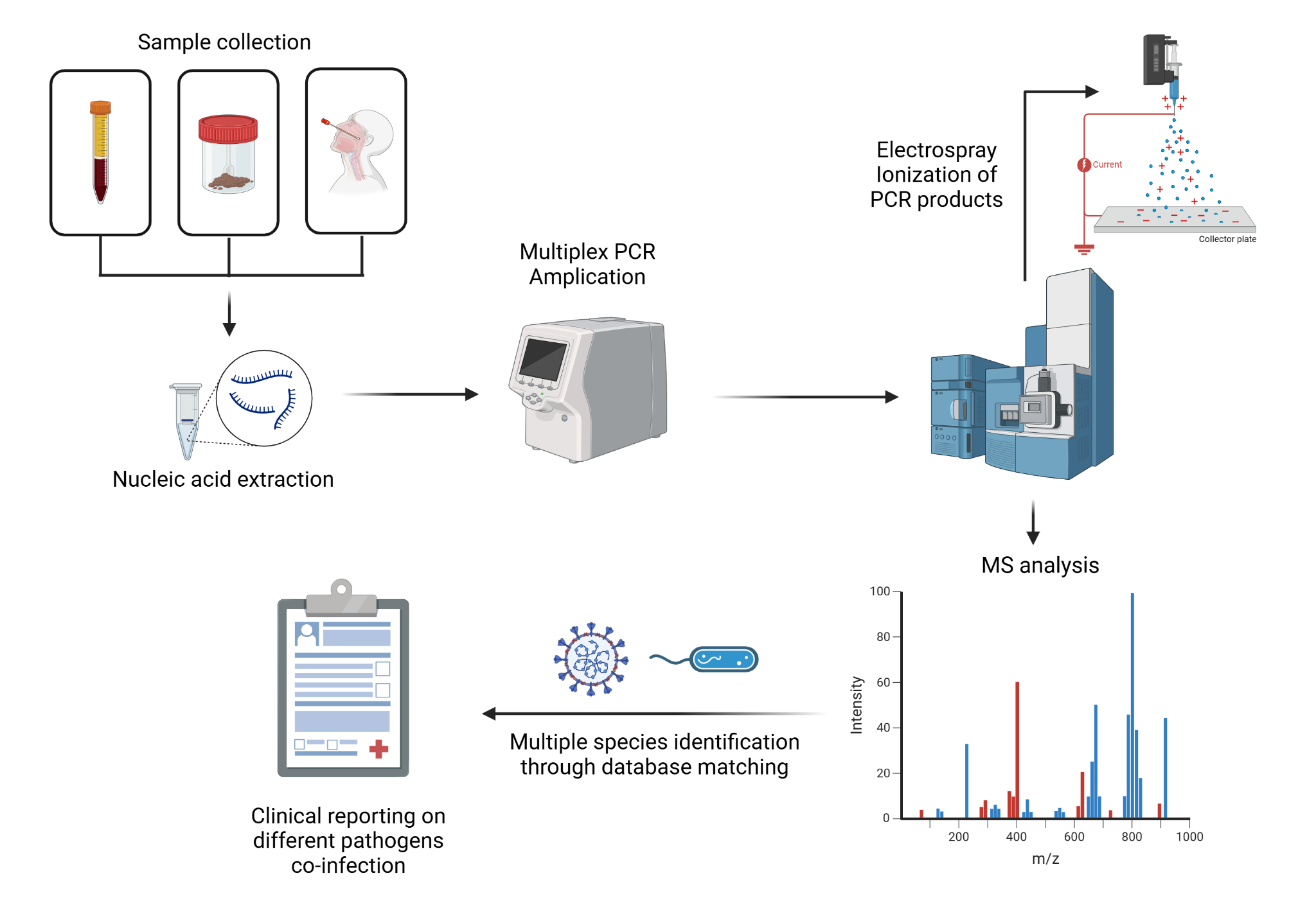
MS was used to detect PCR-amplified genomic fragments of viruses, such asthe hepatitis B virus (HBV), demonstrating its efficiency as an alternative to traditional gel electrophoresis. While the introduction of real-time PCR temporarily overshadowed MS in molecular diagnostics, the technology has since regained attention due to its superior sensitivity, precise molecular sizing, and ability to provide nucleotide composition and charge information [69]. Unlike conventional nucleic acid characterization methods, MS allows for high-throughput and multiplexed detection of multiple pathogens simultaneously. This capability has been effectively utilized in identifying human herpesviruses, human papillomaviruses (HPVs), and HBV variants, showcasing its versatility in clinical diagnostics [70]. By enabling the rapid and comprehensive characterization of viral genomes, MS continues to advance the field of molecular virology, enhancing the detection and differentiation of viral infections directly from clinical samples.

## **VI.C. Detection of mixed infectious agents**

MS can detect mixed infections, meaning it can simultaneously identify multiple pathogens in a sample. It is beneficial when dealing with situations where a patient might have more than one infectious agent causing their illness; this capability is increasingly used in clinical microbiology to diagnose complex infections.

MS has emerged as a powerful tool for detecting bloodstream infections, offering rapid and comprehensive identification of *Mycobacterium* species, including *Mycobacterium tuberculosis complex* (MTBC) and *non-tuberculous mycobacteria* (NTM) [71]. This technology surpasses conventional molecular techniques by enabling the simultaneous detection of multiple species and mixed infections, a critical advantage given the diagnostic challenges posed by co-infections. Unlike Sanger sequencing, which often fails to identify mixed infections, nucleotide MALDI-TOF-MS differentiates species based on variations in molecular masses of single-base extension products [72]. This method has been particularly effective in identifying NTM-MTB mixed infections, which are often misdiagnosed as multidrug-resistant tuberculosis (MDR-TB), leading to inappropriate treatment. However, limitations remain, such as difficulties in distinguishing closely related species within the Mycobacterium avium complex (MAC) and the need for expanding panel coverage to include more species. Despite these challenges, the flexibility of nucleotide MALDI-TOF-MS to incorporate new species and its high-throughput capability make it a valuable tool for bloodstream infection diagnostics, improving pathogen identification, and guiding appropriate treatment strategies.

PCR–electrospray ionization MS (PCR-ESI/MS) has revolutionized the detection of bloodstream infections by providing a highly sensitive and broad-range method for identifying nearly all known human pathogens, including bacteria, fungi, mycobacteria, and viruses, directly from clinical specimens [73]. Unlike traditional microbiological methods or genetic-based detection techniques like real-time PCR, which require prior knowledge of the suspected pathogen, PCR-ESI/MS allows for the unbiased identification of both known and unknown microbial agents by analyzing genetic evidence within patient samples. This method is particularly advantageous in cases of polymicrobial infections, where it can detect mixed amplicons and provide a comprehensive profile of the microbial community present in the bloodstream (Figure 3).

  
**Figure 3. Workflow of PCR-Electrospray Ionization Mass Spectrometry (PCR-ESI/MS) for Mixed Infection Detection (Bacterial & Viral Pathogens). *The image was regenerated using Biorender Premium 2025***

The workflow begins with sample collection and nucleic acid extraction, where clinical specimens such as blood, CSF, respiratory swabs, or stool are processed to isolate both bacterial DNA and viral RNA. If RNA viruses are present, reverse transcription converts them into complementary DNA (cDNA) before amplification. Next, multiplex PCR amplification is performed using broad-range primers targeting conserved bacterial (16S rRNA gene) and viral (e.g. M gene in influenza, ORF1ab in coronaviruses) regions, allowing for simultaneous detection of multiple pathogens. The amplified DNA fragments are then subjected to electrospray ionization (ESI), converting them into charged droplets that enter the time-of-flight mass spectrometer (TOF-MS) for analysis. By measuring the mass-to-charge (m/z) ratio, the system generates a unique molecular fingerprint for each pathogen. The spectral data are then compared against a reference pathogen database, enabling the identification of bacterial and viral co-infections as well as antimicrobial resistance genes. This comprehensive detection process enhances diagnostic accuracy in cases of bloodstream infections, particularly in critically ill patients requiring urgent intervention.

Furthermore, PCR-ESI/MS has demonstrated its utility in pathogen discovery, as exemplified by the first U.S. detection of the novel H1N1 influenza virus in 2009, showcasing its potential to identify emerging infectious diseases [74]. By offering high-throughput capabilities, precise genetic characterization, and the ability to detect novel microbial sequences, PCR-ESI/MS represents a powerful tool in bloodstream infection diagnostics, enhancing pathogen surveillance and guiding targeted therapeutic interventions.

MS can be applied to investigating the potential microbial triggers of multiple sclerosis by enabling the identification and characterization of microbial agents associated with disease onset and exacerbations [75]. Various pathogens, including *C. pneumoniae*, *S. aureus*-produced superantigens, and viruses from the *Herpesviridae* and human endogenous retrovirus families, have been linked to multiple sclerosis, although no single causative agent has been confirmed [75]. MS-based proteomics and metabolomics approaches can help decode the epidemiological contribution of these microorganisms by analyzing microbial-derived proteins, metabolites, and immune system interactions in genetically susceptible individuals. Additionally, MS can provide insights into how common infections, such as those affecting the respiratory, gastrointestinal, and urogenital tracts, may modulate neuroimmunological responses and contribute to disease progression. By offering high-throughput, sensitive, and untargeted pathogen detection, MS holds promise for identifying microbial biomarkers of MS, which could aid in developing new treatment strategies and preventing relapses.

MALDI-TOF MS has been successfully applied to identify multiple bacterial species causing a urinary tract infection (UTI) [76]. By utilizing a differential centrifugation approach to process midstream urine samples, bacterial precipitate can be directly identified using MS or after a short-term culture of 3–6 hours when bacterial counts are lower. This method demonstrated 100% consistency with conventional culture-based identification. Furthermore, the DOT-MGA approach for AST exhibited high concordance with the broth microdilution method, accurately predicting antibiotic susceptibility with no major errors. This approach shortens the rapid identification process to as little as 30 minutes and AST to approximately 4 hours, enabling the rapid diagnosis of UTIs and facilitating timely, targeted antimicrobial therapy. By accelerating pathogen identification and susceptibility testing, MS-based methods provide significant clinical advantages in managing UTIs and improving patient outcomes.

# **VII. ADVANTAGES OF MASS SPECTROMETRY IN MICROBIOLOGY**

MS offers several key advantages in microbiology, including rapid identification of microorganisms at the species level, high accuracy, relatively simple sample preparation, high throughput, cost-effectiveness, and the ability to analyze diverse microbial populations directly from clinical samples, making it a valuable tool for routine microbial identification in clinical and research settings compared to traditional methods like phenotypic testing and biochemical assays [10].

MALDI-TOF MS offers significant advantages over traditional microbial identification methods, enhancing both diagnostic accuracy and clinical decision-making. One key benefit is its ability to detect and report rare pathogens that were previously under-recognized, such as *S. lugdunensis* in skin and joint infections and *Aerococcus urinae* in urinary tract infections. These bacteria often resemble more common species under gram staining and are difficult to differentiate using biochemical methods, leading to misidentification or underreporting before the advent of MALDI-TOF MS [9], [10].

Another major advantage is its rapid turnaround time, which is crucial for guiding timely and appropriate treatment. For instance, in a case of sepsis and gastroenteritis caused by *Listeria* monocytogenes, MALDI-TOF MS enabled identification within an hour, allowing a swift switch to targeted antibiotics and successful patient recovery. The method is particularly beneficial for identifying slow-growing anaerobes and clinically significant yeasts. Traditional methods for anaerobic bacteria require extended culture times, while yeast infections in immunocompromised patients demand urgent identification for optimal treatment. MALDI-TOF MS enables rapid identification, allowing clinicians to select appropriate antifungal therapies based on species-specific susceptibility patterns, leading to improved patient outcomes and shorter hospital stays. These advantages make MALDI-TOF MS an indispensable tool in modern microbiology, revolutionizing pathogen identification and clinical management.

# **VIII. CHALLENGES IN IMPLEMENTING MS IN CLINICAL LABORATORIES**

Implementing MS in clinical microbiology labs offers numerous advantages, but it also comes with challenges. These can be categorized into technical, operational, financial, and regulatory challenges (Table 5):

**Table 5: Challenges in implementing MS in clinical labs**

|  |  |  |  |
| --- | --- | --- | --- |
| **Category** | **Challenges** | **Solution** | **References** |
| **1.Technical** | 1. Limited detection capabilities 2. Database limitations 3. Bioinformatics and Data Management 4. Limited applicability in some pathogens | * Integrating MS with molecular techniques * Improving spectral resolution and employing sophisticated computational methods * Collaborating among clinical laboratories, research institutions, and database providers * Utilizing cloud-based platforms * Investing in training programs and online courses of data analysis and interpretation * Enhancing sample preparation methods to increase viral protein concentration * Developing MS-based assays tailored for viral diagnostics that complement existing PCR-based methods | [77]; [78] |
| **2.Operational** | 1. Need for skilled personnel 2. Integration with workflow 3. Infrastructure requirements | * Investing in comprehensive training programs * Collaborating with academic institutions and professional organizations * Implementing standardized protocols * Adopting automation technologies such as automated liquid handling systems can reduce manual labor and minimize errors * Data transfer between MS instruments and laboratory information systems (LIS)/ electronic health records (EHRs) * Selecting compact or benchtop MS models when space is limited * Investing in uninterruptible power supplies (UPS) and climate control systems can ensure consistent operating conditions | [77]; [78]; [79]; [80]; [81] |
| **3. Financial** | 1. High initial cost 2. Cost-effectiveness in low-volume labs | * Leasing agreements * Seeking funding through grants from governmental or private organizations * Collaborative purchasing agreements * Automating time-consuming workflows, such as solid-phase extraction (SPE) | [82]; [83]; [84] |
| **4. Regulatory** | 1. Standardization of protocols 2. Regulatory and Quality control 3. Challenges with ASTs | * Collaborative efforts in developing and adopting standardized procedures among clinical laboratories, professional organizations, and regulatory bodies * Implementing standardized calibration materials and quality control measures such as the use of internal standards labeled with stable isotope * Implementing comprehensive quality management systems that align with regulatory requirements like CLIA and ISO * Regular proficiency testing, participation in external quality assessment programs, and adherence to validated standard operating procedures * Continuous education and training of laboratory personnel * Focusing on developing MALDI-TOF MS * Expanding MS databases to include spectra of resistant strains | [85]; [86]; [87] |

Implementing MS in clinical practice presents several technical challenges that must be addressed to fully harness its diagnostic potential. Key among these are limited detection capabilities, database limitations, bioinformatics and data management issues, and limited applicability to certain pathogens.

MS, particularly MALDI-TOF MS, primarily identifies pathogens based on protein profiles. This phenotypic approach may not detect genetic resistance markers or virulence factors, potentially leading to incomplete diagnostic information. Additionally, in polymicrobial infections, the simultaneous presence of multiple organisms can complicate spectral interpretation, making it challenging to accurately identify all pathogens present. To overcome these limitations, integrating MS with molecular techniques such as PCR or genomic sequencing can enhance the detection of genetic resistance markers and virulence factors, providing a more comprehensive diagnostic profile. Advanced data analysis algorithms and machine learning techniques can deconvolute complex spectra from polymicrobial infections, improving the accuracy of pathogen identification [77].

The effectiveness of MS-based identification relies heavily on comprehensive and up-to-date spectral databases. However, existing databases may lack entries for all clinically relevant or region-specific pathogens, leading to potential misidentification or non-identification. Furthermore, the rapid emergence of new or mutated pathogens necessitates frequent database updates to maintain diagnostic accuracy. Addressing these challenges requires collaborative efforts among clinical laboratories, research institutions, and database providers to regularly update and expand MS databases. Implementing automated pipelines for continuous database updates can ensure timely inclusion of new or emerging pathogens and resistance profiles, thereby enhancing the comprehensiveness and relevance of these databases [77].

The high-throughput nature of MS generates large volumes of complex data, necessitating robust bioinformatics tools for data processing, analysis, and interpretation. Many clinical laboratories may lack the specialized software and computational infrastructure required to manage these datasets effectively. Additionally, there is often a shortage of personnel trained in bioinformatics, further complicating data management and analysis. To mitigate these issues, investing in high-performance computing infrastructure and specialized software solutions is essential for managing and analyzing extensive MS datasets. Training programs in bioinformatics for laboratory personnel can equip staff with the necessary skills for data analysis and interpretation, ensuring accurate and efficient data handling [78].

While MS has proven effective for bacterial identification, its application in detecting certain pathogens, such as viruses, remains limited. Viral diagnostics often rely on nucleic acid-based methods like PCR, as viruses lack the abundant protein profiles necessary for MS detection. Additionally, MS may struggle to detect specific antimicrobial resistance genes or mutations, which are more readily identified through molecular techniques. Enhancing sample preparation methods to increase viral protein concentrations can improve MS detection of viral pathogens. Developing MS-based assays tailored for viral diagnostics can complement existing PCR-based methods, providing a more comprehensive diagnostic toolkit. Integrating MS with techniques like PCR or sequencing can enhance the detection of antimicrobial resistance genes or specific mutations, allowing for rapid identification of resistance profiles and aiding in the selection of appropriate therapeutic strategies [77].

While advancements in MS have addressed many technical challenges, its integration into clinical laboratories also presents several operational hurdles. These challenges primarily revolve around the need for highly skilled personnel, workflow integration difficulties, and infrastructure requirements. Unlike conventional diagnostic methods, which are often fully automated and standardized, MS demands specialized expertise and tailored laboratory settings, making its adoption in routine clinical practice complex.

One of the most pressing operational challenges in implementing MS is the requirement for highly trained personnel. Unlike traditional diagnostic platforms such as EIA or automated biochemical analyzers, MS-based workflows involve multiple intricate steps, including sample preparation, instrument calibration, method optimization, and data interpretation. Laboratory personnel must possess specialized training in handling mass spectrometers, troubleshooting technical issues, and ensuring quality control [77]. Moreover, the complexity of MS methodologies necessitates continuous education to keep pace with evolving instrumentation and software updates. In settings with high staff turnover, maintaining expertise in MS can be particularly challenging, potentially affecting the consistency and reliability of test results. Addressing this issue requires investment in comprehensive training programs that cover both fundamental and advanced MS applications. Collaborations between clinical laboratories, academic institutions, and professional organizations can facilitate access to standardized training modules, ensuring that personnel remain proficient in MS techniques. Additionally, simplifying workflows through automation—such as pre-calibrated reagent kits and streamlined data processing algorithms—can reduce the burden on laboratory staff and minimize the likelihood of technical errors [88].

Seamlessly integrating MS into existing laboratory workflows presents another major challenge. Many clinical laboratories operate with highly standardized and automated systems, whereas MS-based workflows often require manual interventions, particularly in sample preparation and instrument calibration. Transitioning from conventional diagnostic methods to MS may lead to workflow disruptions, requiring substantial adjustments in laboratory protocols and personnel training. Additionally, integrating MS-generated data with laboratory information systems (LIS) and electronic health records (EHRs) remains a technical hurdle, as many existing LIS platforms are not optimized for handling MS-generated spectral data, necessitating custom software solutions and IT infrastructure modifications [88]. To facilitate smoother integration, laboratories should consider implementing automation technologies that minimize manual handling of samples and reduce turnaround times. Automated liquid handling systems and robotic sample processors can streamline sample preparation, while advancements in software development now allow for direct integration of MS data into LIS/EHR systems, reducing transcription errors and improving result accessibility. Several studies have demonstrated the benefits of automated sample preparation systems in enhancing workflow efficiency and ensuring reproducibility in MS-based assays [79]. Adopting such solutions can improve operational efficiency while preserving the high analytical sensitivity of MS-based diagnostics.

Beyond personnel and workflow considerations, MS instruments also impose specific infrastructure requirements that may not be readily met by all clinical laboratories. Unlike conventional diagnostic instruments, which can often function in standard laboratory conditions, MS systems require carefully controlled environmental settings, including stable temperature and humidity levels, to maintain optimal performance. Additionally, the need for reliable power supplies is critical, as power fluctuations or outages can lead to instrument malfunctions, resulting in data loss and operational downtime. For laboratories operating in older facilities or resource-limited settings, establishing an MS-compatible infrastructure can be costly and logistically challenging [80]. Overcoming these challenges requires strategic planning in laboratory design, ensuring that environmental controls such as temperature regulation and uninterruptible power supplies (UPS) are in place to maintain instrument stability. Laboratories with space constraints may opt for benchtop or compact MS models that offer comparable analytical performance without requiring extensive facility modifications. Moreover, institutions investing in MS should consider phased implementation strategies, starting with limited-scale pilot programs to assess feasibility before full-scale integration [81]. By addressing these infrastructure-related concerns, laboratories can create an environment that supports the successful deployment of MS while maintaining efficiency in routine diagnostic operations.

Although technical and operational constraints, including workflow integration and people training, impede the use of MS in clinical laboratories, financial reasons frequently represent the most significant obstacle. The substantial initial expenditure necessary for MS devices, along with the difficulties in sustaining cost-effectiveness in low-volume facilities, renders financial planning a crucial element in assessing implementation viability. For numerous institutions, the choice to implement MS-based diagnostics depends not only on its technical excellence but also on its economic feasibility.

The acquisition of MS instruments represents a substantial capital investment, with costs ranging from $200,000 to $500,000 depending on the complexity and functionality of the system. In addition to the instrument itself, financial outlays extend to specialized infrastructure, maintenance contracts, and quality control reagents, further increasing the economic burden. Unlike traditional diagnostic methods such as immunoassays, which often utilize pre-existing laboratory equipment, MS requires dedicated resources to ensure optimal performance, including environmental controls and calibration standards. For laboratories operating under constrained budgets, these upfront expenses can be prohibitive, limiting access to MS technology despite its diagnostic advantages. To mitigate these financial constraints, alternative funding strategies must be explored. Leasing agreements offer a practical solution by distributing costs over time, thereby reducing the immediate financial burden on laboratories. Additionally, government and private-sector grants can serve as financial support mechanisms, enabling institutions to invest in cutting-edge diagnostic technologies without incurring excessive debt. Collaborative purchasing agreements between multiple healthcare facilities may also prove beneficial, allowing institutions to share the costs associated with acquiring and maintaining MS instruments. Such financial models have been successfully implemented in research consortia and large hospital networks, demonstrating their feasibility in clinical practice [82].

Even in laboratories that secure funding for MS implementation, maintaining cost-effectiveness remains a concern, particularly in low-volume settings. The high operational costs associated with MS, including consumables, calibration reagents, and maintenance, can outweigh the benefits when sample throughput is limited. Unlike large reference laboratories that process thousands of specimens daily, smaller institutions may struggle to justify the investment in MS technology, as the cost per test remains high when sample numbers are low [83]. To enhance financial sustainability, automation can be leveraged to improve workflow efficiency and optimize resource utilization. Implementing automated sample preparation techniques, such as solid-phase extraction (SPE), can significantly reduce labor costs and minimize reagent waste, thereby improving the cost-effectiveness of MS-based testing [84]. Additionally, outsourcing MS-based testing to centralized laboratories may be a viable option for low-volume institutions, allowing them to benefit from the technology without the need for direct infrastructure investment. Such a model has been successfully adopted in regions where centralized reference laboratories provide specialized testing services to multiple healthcare facilities, ensuring broader access to advanced diagnostics while maintaining economic feasibility.

Last but not least, regulatory compliance and quality control present significant challenges in the clinical adoption of MS. Unlike conventional diagnostic methods, which operate within well-defined regulatory frameworks, MS requires the development of standardized protocols, rigorous quality assurance systems, and validated approaches for ASTs. The absence of universally accepted guidelines complicates accreditation processes and increases the risk of variability in diagnostic outcomes, emphasizing the need for regulatory harmonization to ensure consistency and reliability across clinical laboratories.

One of the primary regulatory challenges in MS implementation is the lack of globally standardized protocols for sample preparation, data acquisition, and spectral analysis. Unlike conventional diagnostic platforms, where reagent kits and automated workflows ensure uniformity, MS-based methods often involve custom-developed protocols that vary across laboratories. This variability can lead to inconsistencies in test results, affecting the reliability of patient diagnoses and limiting inter-laboratory comparability [85]. To mitigate these challenges, collaborative efforts among clinical laboratories, professional organizations, and regulatory bodies are essential. Establishing consensus guidelines for MS-based diagnostics can enhance reproducibility and ensure compliance with industry standards. Additionally, the adoption of stable isotope-labeled internal standards has been shown to improve measurement accuracy and comparability across laboratories, making it a viable approach for standardization [86]. As more laboratories incorporate MS into routine diagnostics, regulatory agencies such as the Clinical Laboratory Improvement Amendments (CLIA) and the International Organization for Standardization (ISO) must play an active role in defining best practices and accreditation criteria.

Beyond standardization, maintaining compliance with regulatory frameworks and ensuring consistent quality control remain significant hurdles. Unlike traditional assays, MS-based workflows involve complex sample processing, instrument calibration, and spectral interpretation, all of which require stringent validation. Regulatory agencies impose rigorous accreditation requirements, but the absence of universal MS-specific guidelines complicates compliance efforts. A study highlighted that while MS offers unparalleled sensitivity and specificity, laboratories often struggle with quality assurance due to the complexity of method validation and performance monitoring [80]. Laboratories must establish extensive quality management systems that comply with regulatory standards to address these challenges. Engagement in external quality evaluation programs and proficiency testing helps substantiate assay performance and guarantee result consistency. Furthermore, continuous education and training for laboratory staff are essential for sustaining proficiency in MS applications, especially in clinical settings where accuracy is critical. By following these principles, laboratories can fulfill regulatory requirements and improve the dependability of MS-based diagnostics.

While MS has significantly advanced microbial identification, its application in AST remains an area of ongoing development. Conventional AST methods, such as broth microdilution and disk diffusion, provide direct susceptibility data but are time-consuming. MS, particularly MALDI-TOF MS, offers a rapid alternative; however, its ability to detect resistance mechanisms is still evolving. The primary challenge lies in the phenotypic nature of MS, which focuses on protein expression rather than genetic determinants of resistance. A review on MS-based AST highlighted that while MALDI-TOF MS can detect certain resistance-associated proteins, its accuracy is limited for identifying resistance genes and mutations without complementary molecular techniques [22]. Research is now focused on developing quick resistance detection methods to enhance the utilization of MS in ASTs. A promising approach is using MS to assess β-lactamase activity, facilitating the rapid detection of β-lactam-resistant bacteria within hours. Furthermore, the incorporation of MS with molecular approaches, such PCR or whole-genome sequencing, can improve resistance profiling by identifying resistance genes in conjunction with phenotypic markers. Augmenting MS spectral databases with resistant strain profiles would enhance accuracy, allowing laboratories to more effectively discern resistance patterns in clinical isolates. Investing in these breakthroughs aids in identification and informs the selection of antimicrobial therapy.

The successful implementation of MS in clinical laboratories is hindered by a range of technical, operational, financial, and regulatory challenges that must be systematically addressed to fully unlock its diagnostic potential. Overcoming these barriers requires a collaborative, multidisciplinary effort involving clinical researchers, regulatory agencies, and industry stakeholders to refine methodologies, streamline workflows, and enhance accessibility. Advancements in cost-effective MS technologies, automation, and bioinformatics-driven solutions will be crucial in facilitating the seamless integration of MS into routine diagnostics. As innovations continue to emerge, addressing these challenges will be essential for ensuring the widespread adoption of MS in clinical microbiology, ultimately enabling more precise, rapid, and comprehensive diagnostic capabilities that enhance patient care and strengthen public health systems.

# **REFERENCES**

[1] E. Garg and M. Zubair, “Mass Spectrometer,” in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: Feb. 01, 2025. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK589702/

[2] J. Hrabák, E. Chudácková, and R. Walková, “Matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis,” *Clin Microbiol Rev*, vol. 26, no. 1, pp. 103–114, Jan. 2013, doi: 10.1128/CMR.00058-12.

[3] A. Berisha *et al.*, “A comprehensive high-resolution mass spectrometry approach for characterization of metabolites by combination of ambient ionization, chromatography and imaging methods,” *Rapid Commun Mass Spectrom*, vol. 28, no. 16, pp. 1779–1791, Aug. 2014, doi: 10.1002/rcm.6960.

[4] P. Seng, J.-M. Rolain, P. E. Fournier, B. La Scola, M. Drancourt, and D. Raoult, “MALDI-TOF-mass spectrometry applications in clinical microbiology,” *Future Microbiol*, vol. 5, no. 11, pp. 1733–1754, Nov. 2010, doi: 10.2217/fmb.10.127.

[5] T.-Y. Hou, C. Chiang-Ni, and S.-H. Teng, “Current status of MALDI-TOF mass spectrometry in clinical microbiology,” *J Food Drug Anal*, vol. 27, no. 2, pp. 404–414, Apr. 2019, doi: 10.1016/j.jfda.2019.01.001.

[6] M. Litterio *et al.*, “Comparison of two MALDI-TOF MS systems for the identification of clinically relevant anaerobic bacteria in Argentina,” *Revista Argentina de Microbiología*, vol. 56, no. 1, pp. 33–61, Jan. 2024, doi: 10.1016/j.ram.2023.12.001.

[7] A. Panda, S. Kurapati, J. C. Samantaray, A. Srinivasan, and S. Khalil, “MALDI-TOF mass spectrometry proteomic based identification of clinical bacterial isolates,” *Indian J Med Res*, vol. 140, no. 6, pp. 770–777, Dec. 2014.

[8] A. Croxatto, G. Prod’hom, and G. Greub, “Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology,” *FEMS Microbiol Rev*, vol. 36, no. 2, pp. 380–407, Mar. 2012, doi: 10.1111/j.1574-6976.2011.00298.x.

[9] N. Singhal, M. Kumar, P. K. Kanaujia, and J. S. Virdi, “MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis,” *Front. Microbiol.*, vol. 6, Aug. 2015, doi: 10.3389/fmicb.2015.00791.

[10] A. E. Clark, E. J. Kaleta, A. Arora, and D. M. Wolk, “Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology,” *Clin Microbiol Rev*, vol. 26, no. 3, pp. 547–603, Jul. 2013, doi: 10.1128/CMR.00072-12.

[11] M. J. Donohue, A. W. Smallwood, S. Pfaller, M. Rodgers, and J. A. Shoemaker, “The development of a matrix-assisted laser desorption/ionization mass spectrometry-based method for the protein fingerprinting and identification of Aeromonas species using whole cells,” *J Microbiol Methods*, vol. 65, no. 3, pp. 380–389, Jun. 2006, doi: 10.1016/j.mimet.2005.08.005.

[12] T. Spanu *et al.*, “Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry in comparison to rpoB gene sequencing for species identification of bloodstream infection staphylococcal isolates,” *Clin Microbiol Infect*, vol. 17, no. 1, pp. 44–49, Jan. 2011, doi: 10.1111/j.1469-0691.2010.03181.x.

[13] P. Lasch *et al.*, “A MALDI-ToF mass spectrometry database for identification and classification of highly pathogenic bacteria,” *Sci Data*, vol. 12, no. 1, p. 187, Jan. 2025, doi: 10.1038/s41597-025-04504-z.

[14] A. Cherkaoui *et al.*, “Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level,” *J Clin Microbiol*, vol. 48, no. 4, pp. 1169–1175, Apr. 2010, doi: 10.1128/JCM.01881-09.

[15] K. Dichtl *et al.*, “A head-to-head comparison of three MALDI-TOF mass spectrometry systems with 16S rRNA gene sequencing,” *J Clin Microbiol*, vol. 61, no. 10, p. e0191322, Oct. 2023, doi: 10.1128/jcm.01913-22.

[16] bioMérieux, “VITEK® MS,” bioMérieux Website. Accessed: Feb. 21, 2025. [Online]. Available: https://www.biomerieux.com/corp/en/our-offer/clinical-products/vitek-ms.html

[17] Bruker, “Microbial Identification,” The MALDI Biotyper® - a revolution in microbiology. Accessed: Feb. 21, 2025. [Online]. Available: https://www.bruker.com/en/products-and-solutions/microbiology-and-diagnostics/microbial-identification.html

[18] Shimadzu Cooperation, “AXIMA Microorganism Identification System,” AXIMA Microorganism Identification System. Accessed: Feb. 21, 2025. [Online]. Available: https://www.shimadzu.com/an/products/maldi/ms-systems/axima-microorganism-identification-system/index.html

[19] Zybio, “EXS 2600\_Zybio,” EXS 2600 Mass Spectrometry System. Accessed: Feb. 21, 2025. [Online]. Available: https://www.zybio.com/productxq/40/20/EXS2600.html

[20] T. R. Sandrin, J. E. Goldstein, and S. Schumaker, “MALDI TOF MS profiling of bacteria at the strain level: a review,” *Mass Spectrom Rev*, vol. 32, no. 3, pp. 188–217, 2013, doi: 10.1002/mas.21359.

[21] K. Teramoto *et al.*, “Phylogenetic classification of Pseudomonas putida strains by MALDI-MS using ribosomal subunit proteins as biomarkers,” *Anal Chem*, vol. 79, no. 22, pp. 8712–8719, Nov. 2007, doi: 10.1021/ac701905r.

[22] W. Florio, L. Baldeschi, C. Rizzato, A. Tavanti, E. Ghelardi, and A. Lupetti, “Detection of Antibiotic-Resistance by MALDI-TOF Mass Spectrometry: An Expanding Area,” *Front. Cell. Infect. Microbiol.*, vol. 10, Nov. 2020, doi: 10.3389/fcimb.2020.572909.

[23] G. Vrioni, C. Tsiamis, G. Oikonomidis, K. Theodoridou, V. Kapsimali, and A. Tsakris, “MALDI-TOF mass spectrometry technology for detecting biomarkers of antimicrobial resistance: current achievements and future perspectives,” *Ann Transl Med*, vol. 6, no. 12, p. 240, Jun. 2018, doi: 10.21037/atm.2018.06.28.

[24] M. L. Faron, N. A. Ledeboer, and B. W. Buchan, “Resistance Mechanisms, Epidemiology, and Approaches to Screening for Vancomycin-Resistant Enterococcus in the Health Care Setting,” *J Clin Microbiol*, vol. 54, no. 10, pp. 2436–2447, Oct. 2016, doi: 10.1128/JCM.00211-16.

[25] C. G. Carvalhaes *et al.*, “Detection of carbapenemase activity directly from blood culture vials using MALDI-TOF MS: a quick answer for the right decision,” *J Antimicrob Chemother*, vol. 69, no. 8, pp. 2132–2136, Aug. 2014, doi: 10.1093/jac/dku094.

[26] E. R. Shaidullina *et al.*, “Detection of carbapenemase-producing Enterobacterales by means of matrix-assisted laser desorption ionization time-of-flight mass spectrometry with ertapenem susceptibility-testing disks as source of carbapenem substrate,” *Front Microbiol*, vol. 13, p. 1059104, 2022, doi: 10.3389/fmicb.2022.1059104.

[27] D. E. Foudraine *et al.*, “Accurate Detection of the Four Most Prevalent Carbapenemases in E. coli and K. pneumoniae by High-Resolution Mass Spectrometry,” *Front Microbiol*, vol. 10, p. 2760, 2019, doi: 10.3389/fmicb.2019.02760.

[28] A. van der Zee *et al.*, “Multi-centre evaluation of real-time multiplex PCR for detection of carbapenemase genes OXA-48, VIM, IMP, NDM and KPC,” *BMC Infect Dis*, vol. 14, p. 27, Jan. 2014, doi: 10.1186/1471-2334-14-27.

[29] M. Yaşar-Duman and F. F. Çilli, “Investigation of the presence of carbapenemases in carbapenem-resistant Klebsiella pneumoniae strains by MALDI-TOF MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) and comparison with real-time PCR method,” *Indian J Med Microbiol*, vol. 39, no. 3, pp. 300–305, Jul. 2021, doi: 10.1016/j.ijmmb.2021.03.013.

[30] J. R. Neil *et al.*, “Rapid MRSA detection via tandem mass spectrometry of the intact 80 kDa PBP2a resistance protein,” *Sci Rep*, vol. 11, no. 1, p. 18309, Sep. 2021, doi: 10.1038/s41598-021-97844-w.

[31] I. D. Nix *et al.*, “Detection of Methicillin Resistance in Staphylococcus aureus From Agar Cultures and Directly From Positive Blood Cultures Using MALDI-TOF Mass Spectrometry-Based Direct-on-Target Microdroplet Growth Assay,” *Front Microbiol*, vol. 11, p. 232, 2020, doi: 10.3389/fmicb.2020.00232.

[32] A. Maus, B. Bisha, C. Fagerquist, and F. Basile, “Detection and identification of a protein biomarker in antibiotic-resistant Escherichia coli using intact protein LC offline MALDI-MS and MS/MS,” *J Appl Microbiol*, vol. 128, no. 3, pp. 697–709, Mar. 2020, doi: 10.1111/jam.14507.

[33] S. Al-Amrani, Z. Al-Jabri, A. Al-Zaabi, J. Alshekaili, and M. Al-Khabori, “Proteomics: Concepts and applications in human medicine,” *World J Biol Chem*, vol. 12, no. 5, pp. 57–69, Sep. 2021, doi: 10.4331/wjbc.v12.i5.57.

[34] E. Khodadadi *et al.*, “Proteomic Applications in Antimicrobial Resistance and Clinical Microbiology Studies,” *Infect Drug Resist*, vol. 13, pp. 1785–1806, 2020, doi: 10.2147/IDR.S238446.

[35] F. J. Pérez-Llarena and G. Bou, “Proteomics As a Tool for Studying Bacterial Virulence and Antimicrobial Resistance,” *Front Microbiol*, vol. 7, p. 410, 2016, doi: 10.3389/fmicb.2016.00410.

[36] R. D. Smith *et al.*, “A Novel Lipid-Based MALDI-TOF Assay for the Rapid Detection of Colistin-Resistant Enterobacter Species,” *Microbiol Spectr*, vol. 10, no. 1, pp. e01445-21, doi: 10.1128/spectrum.01445-21.

[37] J. P. Hulme, “Emerging Diagnostics in Clostridioides difficile Infection,” *Int J Mol Sci*, vol. 25, no. 16, p. 8672, Aug. 2024, doi: 10.3390/ijms25168672.

[38] M. H. Wilcox, “Overcoming barriers to effective recognition and diagnosis of *Clostridium difficile* infection,” *Clinical Microbiology and Infection*, vol. 18, pp. 13–20, Jan. 2012, doi: 10.1111/1469-0691.12057.

[39] A. Nakayama, Y. Morinaga, R. Izuno, K. Morikane, and K. Yanagihara, “Evaluation of MALDI-TOF mass spectrometry coupled with ClinProTools as a rapid tool for toxin-producing Clostridioides difficile,” *Journal of Infection and Chemotherapy*, vol. 30, no. 9, pp. 847–852, Sep. 2024, doi: 10.1016/j.jiac.2024.02.024.

[40] A. E. Boyer *et al.*, “Quantitative Mass Spectrometry for Bacterial Protein Toxins — A Sensitive, Specific, High-Throughput Tool for Detection and Diagnosis,” *Molecules*, vol. 16, no. 3, Art. no. 3, Mar. 2011, doi: 10.3390/molecules16032391.

[41] A. Calderaro *et al.*, “Characterization of Clostridioides difficile Strains from an Outbreak Using MALDI-TOF Mass Spectrometry,” *Microorganisms*, vol. 10, no. 7, Art. no. 7, Jul. 2022, doi: 10.3390/microorganisms10071477.

[42] F. Bittar, Z. Ouchenane, F. Smati, D. Raoult, and J.-M. Rolain, “MALDI-TOF-MS for rapid detection of staphylococcal Panton-Valentine leukocidin,” *Int J Antimicrob Agents*, vol. 34, no. 5, pp. 467–470, Nov. 2009, doi: 10.1016/j.ijantimicag.2009.03.017.

[43] C. Ranasinghe and R. J. Akhurst, “Matrix assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) for detecting novel Bt toxins,” *J Invertebr Pathol*, vol. 79, no. 1, pp. 51–58, Jan. 2002, doi: 10.1016/S0022-2011(02)00004-6.

[44] F. Kondo *et al.*, “Identification of Shiga toxins in Shiga toxin-producing Escherichia coli using immunoprecipitation and high-performance liquid chromatography-electrospray ionization mass spectrometry,” *Analyst*, vol. 128, no. 11, pp. 1360–1364, Nov. 2003, doi: 10.1039/B309401D.

[45] C. K. Fagerquist *et al.*, “Top-Down Proteomic Identification of Shiga Toxin 2 Subtypes from Shiga Toxin-Producing Escherichia coli by Matrix-Assisted Laser Desorption Ionization–Tandem Time of Flight Mass Spectrometry,” *Applied and Environmental Microbiology*, vol. 80, no. 9, pp. 2928–2940, May 2014, doi: 10.1128/AEM.04058-13.

[46] C. K. Fagerquist, Y. Shi, and C. E. Dodd, “Toxin and phage production from pathogenic E. coli by antibiotic induction analyzed by chemical reduction, MALDI-TOF-TOF mass spectrometry and top-down proteomic analysis,” *Rapid Commun Mass Spectrom*, vol. 37, no. 10, p. e9505, Mar. 2023, doi: 10.1002/rcm.9505.

[47] J. A. Roberts, R. Norris, D. L. Paterson, and J. H. Martin, “Therapeutic drug monitoring of antimicrobials,” *Br J Clin Pharmacol*, vol. 73, no. 1, pp. 27–36, Jan. 2012, doi: 10.1111/j.1365-2125.2011.04080.x.

[48] M. Vogeser, C. M. Cobbaert, and E. Hämäläinen, “Mass spectrometry in clinical diagnostics | LabLeaders,” Diagnostics. Accessed: Feb. 08, 2025. [Online]. Available: https://lableaders.roche.com/global/en/articles/mass-spectrometry-moving-clinical-diagnostics.html

[49] A. Dasgupta, “Chapter 2 - Immunoassays and Issues With Interference in Therapeutic Drug Monitoring,” in *Clinical Challenges in Therapeutic Drug Monitoring*, W. Clarke and A. Dasgupta, Eds., San Diego: Elsevier, 2016, pp. 17–44. doi: 10.1016/B978-0-12-802025-8.00002-7.

[50] M. Manohar and M. A. Marzinke, “Chapter 3 - Application of Chromatography Combined With Mass Spectrometry in Therapeutic Drug Monitoring,” in *Clinical Challenges in Therapeutic Drug Monitoring*, W. Clarke and A. Dasgupta, Eds., San Diego: Elsevier, 2016, pp. 45–70. doi: 10.1016/B978-0-12-802025-8.00003-9.

[51] F. Nomura, S. Tsuchida, S. Murata, M. Satoh, and K. Matsushita, “Mass spectrometry-based microbiological testing for blood stream infection,” *Clinical Proteomics*, vol. 17, no. 1, p. 14, May 2020, doi: 10.1186/s12014-020-09278-7.

[52] W. Lu *et al.*, “An LC-MS/MS method for the simultaneous determination of 18 antibacterial drugs in human plasma and its application in therapeutic drug monitoring,” *Front Pharmacol*, vol. 13, p. 1044234, Nov. 2022, doi: 10.3389/fphar.2022.1044234.

[53] E. Myers and E. Dodds Ashley, “Antifungal Drug Therapeutic Monitoring: What are the Issues?,” *Curr Clin Micro Rpt*, vol. 2, no. 2, pp. 55–66, Jun. 2015, doi: 10.1007/s40588-015-0019-x.

[54] S. Barco *et al.*, “A liquid chromatography-tandem mass spectrometry platform for the routine therapeutic drug monitoring of 14 antibiotics: Application to critically ill pediatric patients,” *J Pharm Biomed Anal*, vol. 186, p. 113273, Jul. 2020, doi: 10.1016/j.jpba.2020.113273.

[55] C. Feliu *et al.*, “Quantification of 15 Antibiotics Widely Used in the Critical Care Unit with a LC-MS/MS System: An Easy Method to Perform a Daily Therapeutic Drug Monitoring,” *Pharmaceuticals*, vol. 14, no. 12, Art. no. 12, Dec. 2021, doi: 10.3390/ph14121214.

[56] H. Sun, H. Xing, X. Tian, X. Zhang, J. Yang, and P. Wang, “UPLC-MS/MS Method for Simultaneous Determination of 14 Antimicrobials in Human Plasma and Cerebrospinal Fluid: Application to Therapeutic Drug Monitoring,” *J Anal Methods Chem*, vol. 2022, p. 7048605, 2022, doi: 10.1155/2022/7048605.

[57] T. Khromov, G. H. Dihazi, P. Brockmeyer, A. Fischer, and F. Streit, “24/7 Therapeutic Drug Monitoring of Beta-Lactam Antibiotics with CLAM-2000,” *Antibiotics (Basel)*, vol. 12, no. 10, p. 1526, Oct. 2023, doi: 10.3390/antibiotics12101526.

[58] L. Baietto *et al.*, “Development, Validation, and Routine Application of a High-Performance Liquid Chromatography Method Coupled with a Single Mass Detector for Quantification of Itraconazole, Voriconazole, and Posaconazole in Human Plasma,” *Antimicrob Agents Chemother*, vol. 54, no. 8, pp. 3408–3413, Aug. 2010, doi: 10.1128/AAC.01807-09.

[59] F. Farowski *et al.*, “Quantitation of Azoles and Echinocandins in Compartments of Peripheral Blood by Liquid Chromatography-Tandem Mass Spectrometry,” *Antimicrob Agents Chemother*, vol. 54, no. 5, pp. 1815–1819, May 2010, doi: 10.1128/AAC.01276-09.

[60] L. A. Decosterd *et al.*, “Multiplex Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry Method for Simultaneous Quantification in Human Plasma of Fluconazole, Itraconazole, Hydroxyitraconazole, Posaconazole, Voriconazole, Voriconazole-N-Oxide, Anidulafungin, and Caspofungin,” *Antimicrob Agents Chemother*, vol. 54, no. 12, pp. 5303–5315, Dec. 2010, doi: 10.1128/AAC.00404-10.

[61] Y. Xiao, Y.-K. Xu, P. Pattengale, M. R. O’Gorman, and X. Fu, “A Rapid High-Performance LC-MS/MS Method for Therapeutic Drug Monitoring of Voriconazole, Posaconazole, Fluconazole, and Itraconazole in Human Serum,” *The Journal of Applied Laboratory Medicine*, vol. 1, no. 6, pp. 626–636, May 2017, doi: 10.1373/jalm.2016.022756.

[62] A. F. Lau, “MALDI-TOF for Fungal Identification,” *Clin Lab Med*, vol. 41, no. 2, pp. 267–283, Jun. 2021, doi: 10.1016/j.cll.2021.03.006.

[63] M. E. Zvezdánova, P. Escribano, J. Guinea, P. Muñoz, D. Rodríguez-Temporal, and B. Rodríguez-Sánchez, “Evaluation of the Vitek Ms system for the identification of filamentous fungi,” *Med Mycol*, vol. 60, no. 4, p. myac027, Apr. 2022, doi: 10.1093/mmy/myac027.

[64] N. Motteu, B. Goemaere, S. Bladt, and A. Packeu, “Implementation of MALDI-TOF Mass Spectrometry to Identify Fungi From the Indoor Environment as an Added Value to the Classical Morphology-Based Identification Tool,” *Front Allergy*, vol. 3, p. 826148, 2022, doi: 10.3389/falgy.2022.826148.

[65] J. Chalupová, M. Raus, M. Sedlářová, and M. Sebela, “Identification of fungal microorganisms by MALDI-TOF mass spectrometry,” *Biotechnol Adv*, vol. 32, no. 1, pp. 230–241, 2014, doi: 10.1016/j.biotechadv.2013.11.002.

[66] M. Y. Ashfaq, D. A. Da’na, and M. A. Al-Ghouti, “Application of MALDI-TOF MS for identification of environmental bacteria: A review,” *J Environ Manage*, vol. 305, p. 114359, Mar. 2022, doi: 10.1016/j.jenvman.2021.114359.

[67] C. Firacative, L. Trilles, and W. Meyer, “MALDI-TOF MS enables the rapid identification of the major molecular types within the Cryptococcus neoformans/C. gattii species complex,” *PLoS One*, vol. 7, no. 5, p. e37566, 2012, doi: 10.1371/journal.pone.0037566.

[68] M. Grossegesse, F. Horn, A. Kurth, P. Lasch, A. Nitsche, and J. Doellinger, “vPro-MS enables identification of human-pathogenic viruses from patient samples by untargeted proteomics,” Sep. 27, 2024, *medRxiv*. doi: 10.1101/2024.08.21.24312107.

[69] L. Ganova-Raeva, S. Ramachandran, C. Honisch, J. C. Forbi, X. Zhai, and Y. Khudyakov, “Robust Hepatitis B Virus Genotyping by Mass Spectrometry,” *J Clin Microbiol*, vol. 48, no. 11, pp. 4161–4168, Nov. 2010, doi: 10.1128/JCM.00813-10.

[70] M. I. L. Sjöholm, J. Dillner, and J. Carlson, “Multiplex Detection of Human Herpesviruses from Archival Specimens by Using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry,” *J Clin Microbiol*, vol. 46, no. 2, pp. 540–545, Feb. 2008, doi: 10.1128/JCM.01565-07.

[71] V. Khieu, P. Ananta, O. Kaewprasert, M. Laohaviroj, W. Namwat, and K. Faksri, “Whole-Genome Sequencing Analysis to Identify Infection with Multiple Species of Nontuberculous Mycobacteria,” *Pathogens*, vol. 10, no. 7, p. 879, Jul. 2021, doi: 10.3390/pathogens10070879.

[72] Y. Zhu *et al.*, “Evaluation of nucleotide MALDI-TOF-MS for the identification of Mycobacterium species,” *Front Cell Infect Microbiol*, vol. 14, p. 1335104, 2024, doi: 10.3389/fcimb.2024.1335104.

[73] D. M. Wolk, E. J. Kaleta, and V. H. Wysocki, “PCR-electrospray ionization mass spectrometry: the potential to change infectious disease diagnostics in clinical and public health laboratories,” *J Mol Diagn*, vol. 14, no. 4, pp. 295–304, Jul. 2012, doi: 10.1016/j.jmoldx.2012.02.005.

[74] D. Metzgar *et al.*, “Initial identification and characterization of an emerging zoonotic influenza virus prior to pandemic spread,” *J Clin Microbiol*, vol. 48, no. 11, pp. 4228–4234, Nov. 2010, doi: 10.1128/JCM.01336-10.

[75] M. Marrodan, L. Alessandro, M. F. Farez, and J. Correale, “The role of infections in multiple sclerosis,” *Mult Scler*, vol. 25, no. 7, pp. 891–901, Jun. 2019, doi: 10.1177/1352458518823940.

[76] Z. Liu *et al.*, “Rapid Identification and Drug Sensitivity Test to Urinary Tract Infection Pathogens by DOT-MGA,” *Infect Drug Resist*, vol. 15, pp. 1391–1397, Mar. 2022, doi: 10.2147/IDR.S356045.

[77] S. N. Thomas, D. French, P. J. Jannetto, B. A. Rappold, and W. A. Clarke, “Liquid chromatography–tandem mass spectrometry for clinical diagnostics,” *Nat Rev Methods Primers*, vol. 2, no. 1, p. 96, 2022, doi: 10.1038/s43586-022-00175-x.

[78] S. Cappadona, P. R. Baker, P. R. Cutillas, A. J. R. Heck, and B. van Breukelen, “Current challenges in software solutions for mass spectrometry-based quantitative proteomics,” *Amino Acids*, vol. 43, no. 3, pp. 1087–1108, 2012, doi: 10.1007/s00726-012-1289-8.

[79] D. Anderson, “Mass Spectrometry for Clinical Applications: Challenges and Solutions in the New Decade,” LabX. Accessed: Jan. 31, 2025. [Online]. Available: https://www.labx.com/resources/mass-spectrometry-for-clinical-applications-challenges-and-solutions-in-the-new-decade/279

[80] A. Son *et al.*, “Mass Spectrometry Advancements and Applications for Biomarker Discovery, Diagnostic Innovations, and Personalized Medicine,” *International Journal of Molecular Sciences*, vol. 25, no. 18, Art. no. 18, Jan. 2024, doi: 10.3390/ijms25189880.

[81] V. Zhang, “Bringing Mass Spectrometry to the Clinical Lab,” MedicalLab Management Magazine. Accessed: Jan. 31, 2025. [Online]. Available: https://www.medlabmag.com/article/1208?utm\_source=chatgpt.com

[82] J. M. El-Khoury, “Financial considerations for purchasing a mass spectrometer,” *Yale School of Medicine*, 2018.

[83] P. Dixon, W. Hollingworth, K. Pike, R. Reynolds, M. Stoddart, and A. MacGowan, “Cost-effectiveness of rapid laboratory-based mass-spectrometry diagnosis of bloodstream infection: evidence from the RAPIDO randomised controlled trial,” *BMJ Open*, vol. 11, no. 10, p. e044623, Oct. 2021, doi: 10.1136/bmjopen-2020-044623.

[84] N/A, “Education and Expense: The Barriers to Mass Spectrometry in Clinical Laboratories?,” Analysis & Separations from Technology Networks. Accessed: Jan. 31, 2025. [Online]. Available: http://www.technologynetworks.com/analysis/news/education-and-expense-the-barriers-to-mass-spectrometry-in-clinical-laboratories-193157

[85] H. W. Vesper, G. L. Myers, and W. G. Miller, “Current practices and challenges in the standardization and harmonization of clinical laboratory tests1223,” *The American Journal of Clinical Nutrition*, vol. 104, pp. 907S-912S, Sep. 2016, doi: 10.3945/ajcn.115.110387.

[86] J. L. Bock and J. H. Eckfeldt, “Advances in standardization of laboratory measurement procedures: implications for measuring biomarkers of folate and vitamin B-12 status in NHANES1,” *The American Journal of Clinical Nutrition*, vol. 94, no. 1, p. 332S, Jul. 2011, doi: 10.3945/ajcn.111.013359.

[87] M. Vogeser, “Mass Spectrometry in the Clinical Laboratory—Challenges for Quality Assurance,” vol. 13, pp. 14–19, Jul. 2015.

[88] M. S. Cooke Peter, “Bringing down barriers to give better access to mass spectrometry in the clinical laboratory,” Medical Laboratory Observer. Accessed: Jan. 31, 2025. [Online]. Available: https://www.mlo-online.com/molecular/mdx/article/21274162/bringing-down-barriers-to-give-better-access-to-mass-spectrometry-in-the-clinical-laboratory