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Mass Spectrometry - based Methods for the Identification of Bacteria: A Systematic Review

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Abstract

Traditional methods for bacterial identification have often been limited by the lack of advanced mass spectrometry (MS) assays. The advent of novel MS techniques has the potential to revolutionize bacterial detection and screening. The purpose of this review is to assess and compare various mass spectrometry techniques applied to bacterial detection. We aim to identify the strengths and limitations of each method to understand their effectiveness and potential applications better. This systematic review was conducted following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for observational studies [31]. We performed an extensive search using electronic databases, including PubMed, as well as additional sources relevant to mass spectrometry (MS) and bacterial identification. Keywords used in the search included mass spectrometer, microbiology, bacteria, identification, and detection. Our inclusion criteria focused on original research articles written in English and published between January 2019 and January 2024. We reviewed bibliographies of these articles to ensure comprehensive coverage of relevant studies. The initial search yielded 4,690 studies from PubMed and 1,409 from other databases. After removing duplicates and irrelevant studies (3,425 from PubMed and 3,308 from other sources), 117 full-text studies were assessed for eligibility. Of these, 37 studies were excluded due to their focus on other aspects of microbiology rather than bacterial identification. Additionally, 76 studies were excluded for various reasons, such as methodological limitations or lack of relevance. Ultimately, six studies that specifically addressed the use of MS for bacterial detection and identification were selected for detailed analysis.The review highlights that integrating PCR analysis with mass spectrometry could enhance bacterial identification techniques. Specifically, MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) has demonstrated considerable promise in improving the efficiency and accuracy of bacterial detection in laboratory settings. The combined approach of PCR and MS offers a robust platform for enhancing bacterial identification and could lead to significant advancements in microbiological research and diagnostics.

Keywords: *Mass spectrometry, Techniques, Identification, Bacteria*

1. Introduction

Mass spectrometry (MS) traces its origins to the early 20th century with J.J. Thomson's discovery of the electron and the development of the mass spectrograph [1, 2]. Francis Aston built on this work, creating the first mass spectrometer and advancing isotope research significantly [3]. In the mid-20th century, Wolfgang Paul introduced quadrupole mass filters [4], and William Stephens developed time-of-flight mass spectrometry [5]. Alfred Nier's electron ionization method, introduced in the 1950s, became a standard technique [6].The late 20th century brought major innovations, including John Fenn's electrospray ionization [7] and MALDI (matrix-assisted laser desorption/ionization) by Hillenkamp and Karas [8], which transformed biomolecular analysis. In 1952, it was reported that volatile pyrolysis could be detected using MS [9]. By the 1960s, the combination of pyrolysis and gas-liquid chromatography (Py-GLC) advanced the technology, proving useful for identifying and categorizing pathogenic bacteria and other microbes [9, 10]. Additionally, integra typically consist of peaks ranging from 1,000 to 30,000 m/z, with those between 2 and 20 kDa being most frequently ting a quadrupole mass spectrometer with a Curie-point pyrolizer enabled consistent bacterial fingerprinting [11]. A notable report by Anhalt and Fenselau in 1975, titled "Identification of bacteria applying mass spectrometry," highlighted how hard ionization methods allowed for the identification of bacterial lipids, though with limited ability to differentiate species [12]. The development of

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soft ionization techniques [13] improved microbial identification by focusing on proteins and peptides. Modern methods compare peptide peak profiles and ionized proteins to existing spectral records. Mass spectral signatures used due to their strong signal-to-noise ratio and stability. Research by Ryzhov and Fenselau showed that ribosomal proteins, cold shock proteins, and DNA-binding proteins were prominent in MALDI-TOF MS analyses of Escherichia coli [14]. Mass spectrometry, a powerful analytical tool, determines the mass-to-charge ratio (m/z) of molecules in a sample. Compared to traditional techniques like immunoassays, MS offers enhanced accuracy and the ability to detect multiple targets simultaneously. The increasing robustness and sophistication of MS technology have led to its widespread adoption in various medical fields. Clinical medicine often uses liquid chromatography (LC) combined with tandem mass spectrometry (MS/MS) for applications such as toxicology [15], endocrinology [16], therapeutic drug monitoring [17, 18], newborn screening [19], and proteomics research [20]. Additionally, MALDI imaging MS has enabled label-free, multiplex assessment of molecules within tissue samples, allowing for rapid assessment of surgical tissue specimens [21, 22].In recent years, mass spectrometry has become increasingly important in biology, offering sensitive, high-throughput testing for microbiological applications including environmental analysis, research, and medical diagnostics. This article reviews the range of MS methods applied to bacterial and microorganism identification and their effectiveness in laboratory settings [23]. In environmental and clinical microbiology, a primary goal is the rapid and accurate detection of microorganisms in complex biological samples [24]. Traditional methods for microorganism identification are often slow and labor-intensive. MALDI-TOF MS has emerged as a highly effective tool for identifying microorganisms in bloodstream infections, transforming clinical microbiology with its simplicity, speed, and ease of use [25–30]. Despite its advantages, MALDI-TOF MS has limitations in identifying bacterial susceptibility and resistance. The integration of LC with MS/MS holds promise for addressing these limitations and advancing clinical microbiology research. Novel mass spectrometry assays are crucial for overcoming the challenges posed by slow culture-based methods and the low specificity of traditional biochemical tests. MS offers rapid, precise, and high-throughput identification, enhancing diagnostic accuracy and enabling timely treatment, ultimately improving clinical workflows and patient outcomes. This systematic review focuses on the various MS methods developed for bacterial assessment and their effectiveness in identifying bacteria directly from culture results.

2. Materials and Methods

Study selection

The present systematic overview is carried out following the guidelines for observational studies in epidemiology [31] using PRISMA (Flowchart 1) [32]. A brief overview of the method used is presented in Figure 1. PubMed and other sources were used as the database to identify the records, and keywords (mass spectrometer, microbiology, bacteria, identification, and detection) have been used to search for the sources. Only original, written-in English articles published between January 2019 and January 2024 have been included in the search criteria. In brief, we obtained full-text publications on the MS that were used to assess, identify, and determine whether a bacterium was involved; the authors of the original articles did not request further details.

Data extraction and eligibility criteria

The search strategy involves systematic literature searches in databases like PubMed and Scopus. Inclusion criteria are peer-reviewed articles on mass spectrometry for bacterial identification, published in the last decade. Exclusion criteria include non-English studies and non-relevant methodologies. Data extraction focuses on study design, techniques used, and outcomes reported.For the purpose of assessing each included study, we used a standard procedure that took into account the first author, the year of publication, and the particular type of MS study that had been used to identify bacteria. Following the removal of duplicate articles, titles, abstracts, and contents were evaluated. The study sections reached a consensus regarding the identification of bacteria. After the records were independently reviewed, 4690 studies from PubMed and 1409 research studies from other databases were identified. Duplicate and irrelevant studies (n = 3425 and n = 3308) were excluded. Consequently, of the remaining 117 full-text studies evaluated, 37 were deemed ineligible for having connections to other areas of microbiology. About 76 studies were excluded for various reasons out of the 80 relevant studies that were screened with a focus on bacteria. At last, six articles were chosen based on MS and bacteria (Figure 1, PRISMA flowchart, which illustrates the search and screening process). The first author, MS type used, study design, the aim of the study, bacteria isolated, sample size, and MS technique used were among the information that were extracted. The risk of bias in observational research and the quality testing of the research included in the study have been assessed by the author using the Newcastle-Ottawa Scale. Selection, comparability, and outcome were among the quality elements assessed [33].

Ethical approval

Ethics approval is not needed for this systematic review study because the included data are based on previously published articles, and participant-identifying information will not be disclosed.

3. Results and Discussion

The electronic search selected six full-text studies covering a range of bacterial identification and screening by MS from 2019 to 2024. The progression from the abstracts to the classification of the MS used in the evaluation of bacteria is illustrated in Figure 1. After limiting the analysis to just taking into consideration MS techniques, the collection of completed research was

reduced. The number of abstracts associated with each of those techniques for identifying bacteria was used to categorize them as well. The MS methods for identifying bacteria clearly shifted based on the related total sum of summary information. These 6 studies mentioned the well-known bacteria screening techniques; there were more occurrences in the abstracts, and some of them were clearly recognized in bacteria and included all MS types. Information on the reviewed studies included is presented in Tables 1 and 2. In Table 1, the most studied designs were cross-sectional, resulting in higher bacteria identification when compared with other designs. MALD-TOF-MS was the most common MS. Besides, TPPI-TOF-MS, LC/MS, and GC/MS techniques were used for the identification of bacteria. Also, the number of bacteria samples isolated (sample size), the bacterial strains involved, and the accuracy of each MS used are summarized in Table 2.

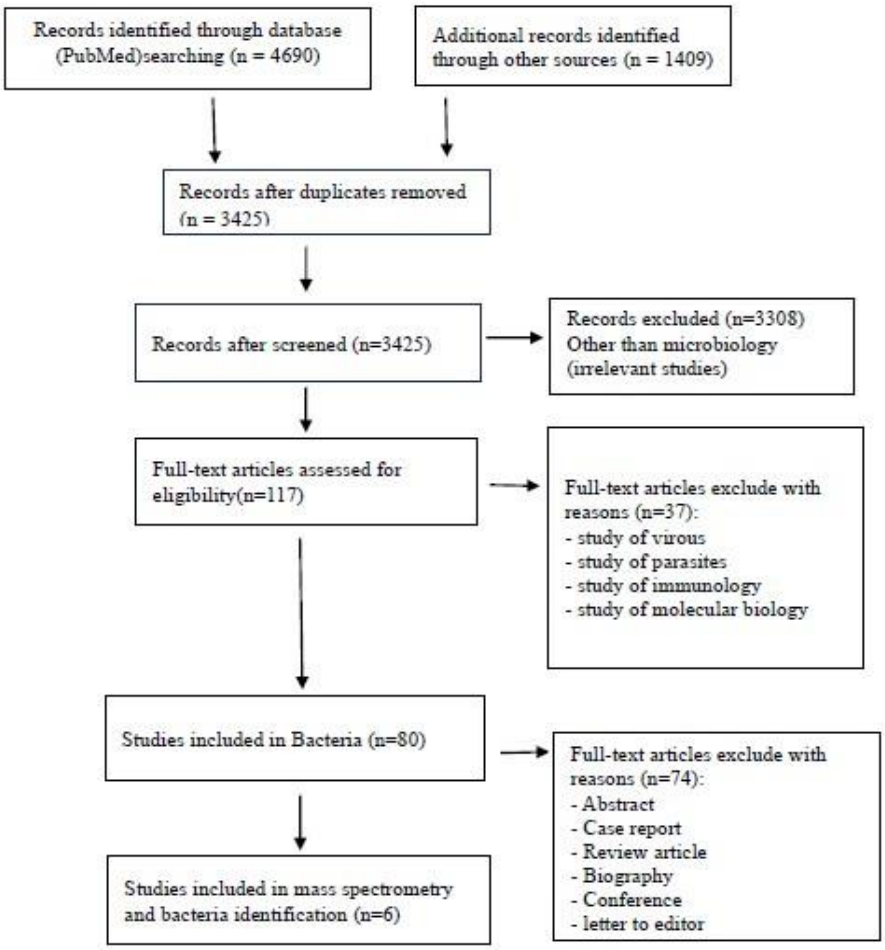

Figure 1: PRISMA flowchart for the included studies in this systematic review

Table 1: The representative examples of mass spectrometer used in the clinical Bacteriology

Instrument type	Study design	Purpose	References
HPPI-TOFMS	Cross-sectional	Detection	Liang et al. (2023) [56]
Microflex LT bench-top	Cross-sectional	Identification	Claire et al. (2020) [57]
Autof MS1000	Prospective cohort	Evaluation, analysis	Qiong et al. (2020) [58]
GCMS-QP2020 NX	Cross-sectional	Identification	Gassiep et al. (2019) [59]
MALDI Biotyper	Cross-sectional	Identification	Fumio et al. (2020) [60]
Vitek MS	Cross-sectional	Identification, analysis	Rosamaris et al. (2023) [61]

Keys: *High-pressure photon ionization time-of-flight mass spectrometry (HPPI-TOFMS); Gas chromatograph mass spectrometer (GCMS); Mass spectrometry (MS); matrix-assisted laser desorption ionization (MALDI).*

Table 2: Baseline characteristics of the studies included for systematic review

Author (year)	*Bacteria isolated*	Samples size	Technique used	Succuss accuracy % (95% CI)
Liang et al. (2023) [56]	Mycobacterium Tuberculosis	435	TPPI-TOF-MS	80.3%
Claire et al. (2020) [57]	Genus Burkholderia	95	MALDI-TOF-MS	99.5%
Qiong et al. (2020) [58]	Different bacteria strains	2342	MALDI-TOF MS	99.7%
Gassiep et al. (2019) [59]	Burkholderia Pseudomallei	250	MALDI-TOF MS	41%
Fumio et al. (2020) [60]	Bloodstream bacteria's	57	MALD-TOF-MS	99.9%
Rosamaris et al. (2023) [61]	Legionella Pneumophila	48	LC/MS & GC/MS	89%

Keys: *High-pressure photon ionization time-of-flight mass spectrometry (HPPI-TOF-MS); Gas chromatograph mass spectrometer (GC-MS); Liquid chromatography (LC); Mass spectrometry (MS); matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS).*

In clinical and medical microbiology, MS techniques for microorganism identification are a great substitute for conventional laboratory methods. In order to identify bacteria, advanced technologies like desorption, electrospray, and ionization have begun to be used recently. Mass spectrometry's direct identification of bacteria has a number of benefits, including quickness and ease of use [34]. Sufficient detection capability and selectivity can only be achieved with careful sample pretreatment processes, but efficient flow should be ensured by minimizing the amount of time needed. It is possible to simplify bacterial indicators using a variety of chromatography-based techniques [35]. Measurable differences in bacterial genomic information can be used by MS methods to facilitate the identification of bacteria. Even though the current generation of PCR assays is quick, sensitive, and specific, it is not possible to use them directly for categorization, particularly when dealing with samples of unknown bacteria. Techniques that combine PCR and MS build on the advantages of both techniques and, occasionally, offer further details that can't be obtained with each method alone [36].The 76 excluded studies often fell short due to factors such as non-peer-reviewed sources, outdated methodologies, or irrelevant focus areas. Common reasons included inadequate sample sizes, lack of specificity in mass spectrometry techniques, or failure to provide clear identification results. These exclusions ensure the review's relevance and methodological rigor.This review concludes with a few clinical applications related to bacterial analysis and some perspectives. It also summarizes the methods of bacteria identification and analysis, including record searches, for the differentiation of MS techniques. By combining immunomagnetic separation with MALDI-MS, Madonna et al. [37] created a quick technique to detect specific bacteria from complex biological study options. In just one hour of analysis, this method could detect an item from a biological buffer in only microliter volumes. Many benefits come with direct analysis of bacteria using MALDI-MS, which include speed, minimal identification restrictions, simplified mass spectra (which show signals from primarily singly charged ions), and resistance to various contaminations. Although only a small portion of the bacterial proteins have been identified, these protein profiles in whole-cell MALDI mass spectra have scientifically distinct characteristics that can be used for distinguishing bacteria among the genus, species, and strain at different levels. Several clinical microbiology centers have used direct evaluations of unaltered bacterial cells with MALDI-TOF MS to distinguish between a variety of bacteria and a subspecies [38]. Since bacteria clearly differ from one another, the protein profiles can be used to differentiate between different species. A number of authors have assessed the efficiency of using MALDI-MS to identify and type bacteria [39, 40–43]. By combining GC-MS and LC-MS, more metabolism protection can be obtained [32]. In their investigation, Smilde et al. [44] discovered a significant amount (93%) of the commercially available metabolites of the in-silicometabolomes of B. subtilis and E. coli. Similar acceptance (95–97%) for S. cerevisiae and the same bacteria required the use of six different analytical techniques [45]. When analyzing complex environmental and clinical samples for carbohydrate biomarkers (like muramic acid), GC-MS/MS can enhance the analysis's particularity and identification limitations. In the numerous reactions tracking modes, this technique requires monitoring the accurate transitions of precursor ions to fragment particles. Using this method, Wunschel et al. [46] investigated the viability of analyzing agar elements linked to Bacillus anthracis spores by applying two variations to the alditol acetate method. When spores were present, these techniques could identify the 3,6-anhydro-l-galactose agar background element. In order to classify and identify bacteria, pyrolysis-MS is commonly applied to get a pyrolusite fingerprint [47], and proteins [48] have all been used for bacterial inequality. The effective implementation of GC-MS for metabolism product identification for bacterial characterization has been suggested [46]. Compounds with a maximum molecular weight of 1000 Da can be analyzed using GC-MS. Nowadays, it is the most popular analytical technique.Ionization methods, biological approaches, and instrumentation (mass and separation analysis) advancements are all going to enhance mass spectrometry's pathogen-analysis abilities [49, 50].For the separation and examination of biological materials like proteins, peptides, nucleotides, and substances, liquid chromatography is combined with mass spectrometry. As a result of its capacity for chromatographic

separation and the capability to combine various separation columns in a flexible manner, this form of separation is most likely best suited for clinical applications in the analysis of microbes. It has been studied how the study preparation techniques influence LC-ESI-MS detection of bacterial proteins isolated from E. coli [51]. Additionally, the impact of differences in protein patterns on the identification of bacteria has been investigated. Lo et al. [52] identified several bacterial species in a single MS assessment using an LC-selective peptide analysis method. If the chosen peptides were correctly extracted within the established opening, the related bacterial species would be recognized. In a single LC-selective peptide analysis experiment, this technique was used to identify pathogens in the bacterial mixes [52]. The limited quantity of available research studies in this present review is a limitation relating to our analysis. Microbiology has an increased body of study evidence available, which may introduce bias. Environmental studies may also be a significant variable, and applying results from bacteria could be less safe. All of these things may need to be considered as well.

4. Conclusions

MS has been shown to be a helpful technique for identifying bacteria by the instruments used to identify them. However, one of its biggest obstacles is detecting complex samples. To address the challenges posed by complex samples, techniques for analyzing samples without pretreatment or following thorough biochemical and chromatographic fractionations have been developed. Rapidity and simplicity of use are two benefits of direct MS-based analyses of bacteria. The MALDI interaction is most useful for detecting bacterial risks in our daily lives or on battlefields using field-portable mass spectrometers. To prevent contamination in bacterial assessments, isolated cultures are typically used, unless data evaluation techniques are used or those that interfere are eliminated. Many chromatography-based techniques (such as HPLC and CE) can be used to simplify the complexity of bacterial markers; while the rate of identification is a little deteriorating, the precision, reliability, and flexibility have been considerably elevated. In the upcoming years, increasing the detection limits for bacterial cells will remain a top priority. Cell enrichment via affinity techniques will thus become more and more crucial. Using affinity techniques in conjunction with top-down protein analysis and selective MS analysis will improve identification accuracy and detection capability. An alternative way to increase sensitivity would be to use MS detection in conjunction with PCR amplification of nucleic acids, particularly when analyzing nonculturable bacteria. Apart from MALDI, a number of novel ionization methods have shown great promise for use in real-time direct MS-based pathogen analyses. These methods include desorption electrospray ionization [53], direct analysis in real time [54], and ESI-assisted laser desorption ionization [55]. More effective instruments for characterizing bacteria can also be made attainable by improvements in other mass spectrometry-related technologies. These technologies consist of high-capacity linear ion traps, ion mobility spectrometry, high-speed and high-resolution LC, and various high-resolution mass analyzers, including Fourier transform ion cyclotron resonance, Fourier transform orbitrap, and time-of-flight mass detectors. These techniques, along with the right equipment, will certainly enhance MS's pathogen analysis capabilities.PCR paired with mass spectrometry (MS) offers a robust platform for bacterial identification by combining the sensitivity of PCR with the detailed profiling of MS. PCR amplifies specific bacterial DNA, ensuring precise target detection, while mass spectrometry, particularly MALDI-TOF-MS, provides detailed protein fingerprints, enhancing identification accuracy. MALDI-TOF-MS excels in rapid, high-throughput analysis with minimal sample preparation. Future research could explore optimizing PCR-MS integration for diverse bacterial strains and refining MALDI-TOF-MS techniques to reduce detection limits and improve resolution. Clinically, this combination could streamline diagnostics, reduce turnaround times, and enable comprehensive microbial profiling, ultimately improving patient outcomes.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

No – funding stated.

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