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The Use of MALDI-TOF Mass Spectrometry Technology in Molecular Analysis of Microbial Pathogenesis

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Abstract: Mass spectrometry is a method of evaluation used to identify which particles compose an example based on the ions' mass spectrum. Mass spectrometers can perform conventional detection and quantitation of target analytes. However, they can also be used for the rapid discovery of bacteria within a medical setting. Matrix-assisted laser desorption/ionization-time of flight mass spectrometer is one of the most prominent MS tools applied in biology, with its robust and accurate recognition of categories and types of a wide variety of Gram-positive and negative microorganisms. Mass spectrometry detection is based on determining a particular range of each kind and matching it with a comprehensive data source within the tool. Today's study describes the history and sample preparation of the MALDI-TOF MS technique. Moreover, the applications of MALDI-TOF MS microbial recognition in the center and the presence of antimicrobial resistance will be presented. Besides, the present restrictions and future use of MALDI-TOF MS in forthcoming daily scientific practice are reviewed. In this review, microorganisms will also be addressed in future clinical applications, primarily using MALDI-TOF MS in microbiology to identify and analyze antibiotic resistance.

Keywords: infection; MALDI-TOF MS; microorganism; microbiology; mass spectrometry.

1. Introduction

The advancement and use of unique microbes recognition methods are vital to establishing one of the most suitable antimicrobial treatments [1]. The principal targets of the microbiological management of patients suspected of having a microbial infection are to recognize the infectious microorganism from the disease's site and determine the antibiotic minimum inhibitory concentration (MIC) to assess the antimicrobial task representative versus the pathogen [2]. These methods are based on the bacterium's pure culture, meaning that this conventional method requires a minimum of 36-48 hours from the medical sample distribution to address the clinical demands [3]. This time around is dramatically raised if the bacterium's development is slow or postponed or the antimicrobial resistance is not useful [4]. For several years medical microbiology has been based on reliable but long-lasting approaches that did not fulfill the moment required for rapid individual administration [5-7]. Hence, molecular

diagnostic methods not needing microbes' culture development might be represented as a benefit over contemporary approaches.

Besides molecular techniques, Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) has been established to recognize many pathogenic microorganisms [8, 9] quickly. Mass spectrometers straight analyze any ionized susceptible organic molecule [10]. Because of its conception, this device has changed the approach to identifying medical laboratories since that is a fast, high-efficiency, low-cost, and useful device [11]. Among the considerable benefits of using MALDI-TOF MS conserves time [12]. This time around is important for individuals with an underlying autoimmune condition or immunocompromised patients [13]. In this review, the history of the MALDI-TOF MS method and the preparation of the sample is explained. After that, the applications of MALDI-TOF MS microbial recognition in the clinic and antimicrobial resistance exist. Furthermore, it attempted to discuss the restrictions and possible use of MALDI-TOF MS in upcoming daily medical techniques.

2. MALDI-TOF MS and Sample Preparation

MS is utilized to identify the m/z ratio, and MALDI-TOF MS supplies fast, precise, and sensitive spectra of the bio-analytes within a sample [14]. MALDI is an ionization technique in which the matrix captivates energy from ultraviolet lasers to generate ions from large molecules with minimal fragmentation. The ratio of m/z can be calculated by the TOF of the ions, which the detector measure TOF of ions to estimate masses of ions (Fig. 1). The a-Cyano-4-hydroxycinnamic acid (HCCA) and 2, 5-dihydroxybenzoic acid is MALDI matrices used for several microbial studies [15]. For microorganism recognition, positively charged peptides or proteins with a molecular weight between 2000 and 20,000 m/z can be used [16]. The sequence and size of ribosomal proteins are highly conserved among diverse bacterial species and classify individual types of bacteria [17]. Unique mass peaks are used to detect microorganisms and provide useful information for bacteria fingerprinting [18]. These findings indicate that microorganisms can be classified rapidly, accurately, efficiently, and reliably. The innovative MS system has been designed with a highly automated operational workflow and analysis process.

Sample preparation for clinical MALDI-TOF MS applications is supposed to be appropriate and straightforward for transferring a small amount of biomaterial from the culture plate to the MALDI sample plate [19]. However, it has been demonstrated that the essence of the culture medium used can affect efficiency [20]. Furthermore, simple washing steps can solve these dysfunctions when involving sampling from tangible culture media [21]. Pre-purification is compulsory for the direct examination of liquid cultures or liquid clinical specimens. The most frequently requires a short pre-culture on rich media, filtration, or, most popular, combined centrifugation and washing steps [22-24]. Sufficient bacterial identification was reported by MALDI-TOF MS of positive blood cultures and urine samples using simple pre-analytical tools [25]. Cell inactivation is needed in the case of elevated biohazard-level organisms. Also, various, mostly organic solvents or short-term heat inactivation are most common [26]. There may also be a need for mechanical lyses and protein extraction [27, 28]. Trifluoroacetic acid is another chemical used effectively to rupture cells and, in this case, coincide with protein precipitation [29]. Efficiently, all manipulations around the sample preparation should be automated, but the techniques that facilitate all the handlings listed are not yet commercially available [30, 31]. Only when such instruments become available can

one begin to consider more complex liquid handling systems that may, for example, also require specific affinity binding to sample holders of a particular protein or microbial cells [32]. With automation, the detection limit of ~105 cells per sample could be reduced to single cells [33]. Thus, respective techniques have been developed, but routine diagnostics are far from being implemented (Figure 1).

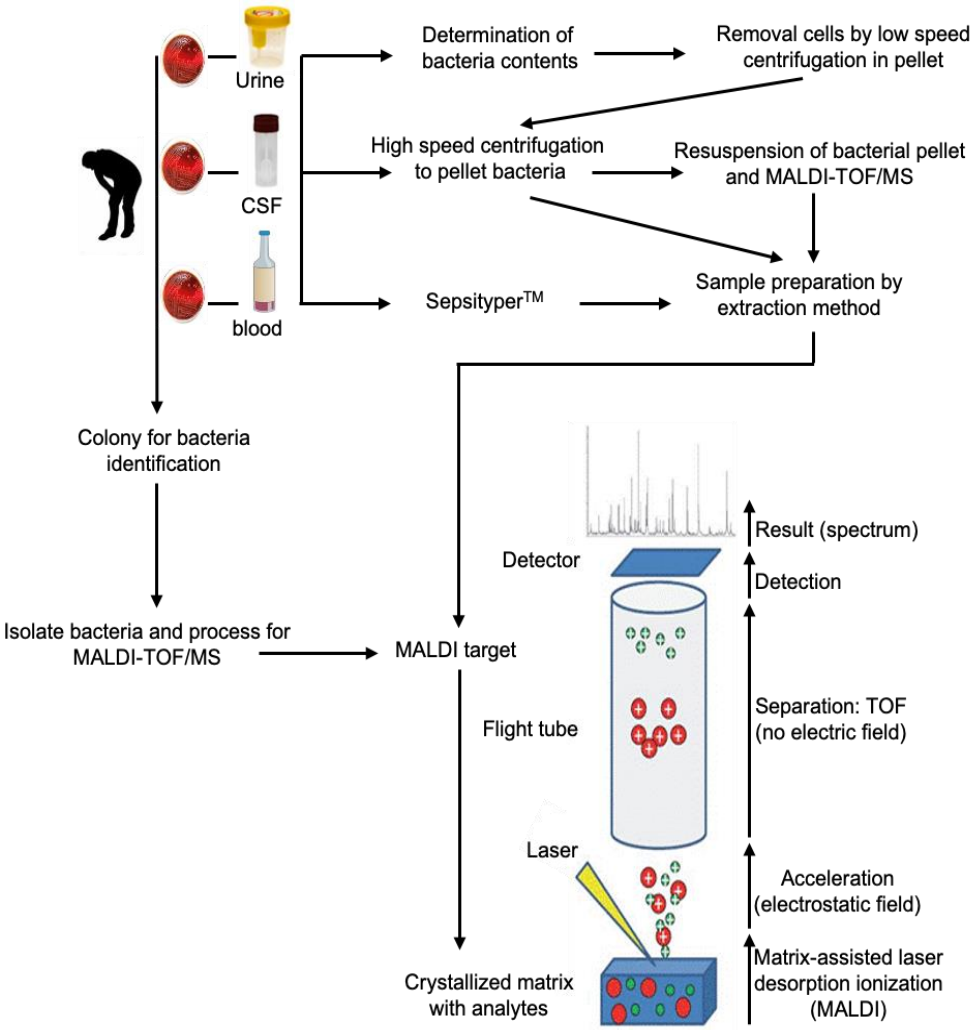

Figure 1. Position of MALDI-TOF MS in the workflow of the clinical microbiology laboratory, including the current options for analysis of bacteria directly from patient specimens. Figures are originally created by biorender.

3. MALDI-TOF MS for Therapeutic Proteomics

MALDI-TOF MS-based methods have been accepted for clinical practice, microbial research laboratories, and pharmaceuticals (Figure 2). Furthermore, serum protein profiling by MALDI-TOF MS will likely go into routine clinical.

3.1. Applications for microbiology.

Quick and accurate microbes detection is critical in microbiology to treat illness medical diagnosis and provide prompt and effective antibacterial therapy [34].

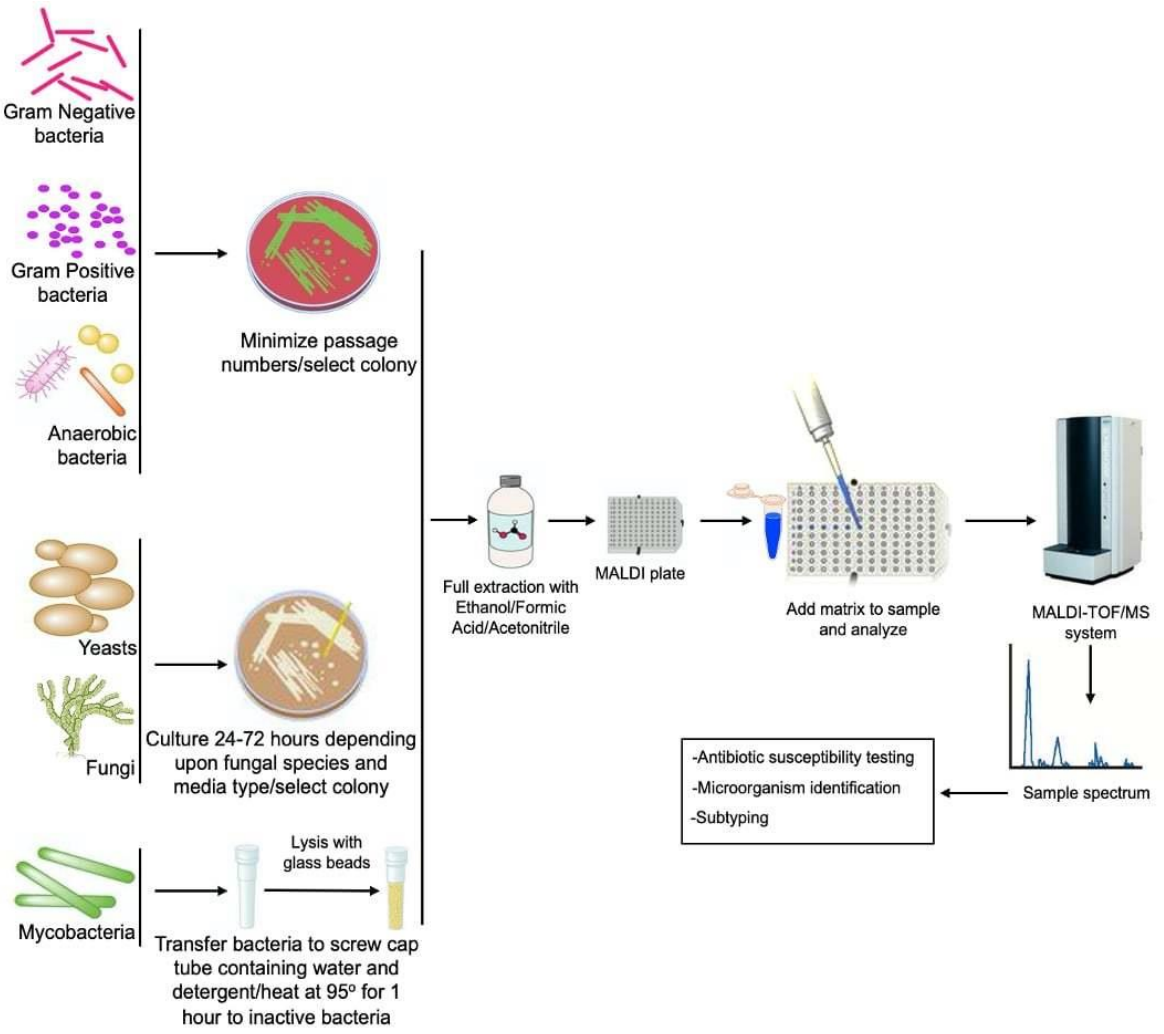

Figure 2. The overall MALDI-TOF MS sample preparations for use with different groups of microorganisms.

3.1.1. Gram-negative bacteria recognition.

MALDI-TOF MS has been used by individual researchers over the past few years, evaluating its ability to distinguish various microbial categories within Gram-negative rods, including *Escherichia coli* and diverse other families of *Enterobacteriaceae* [35, 36]. Gram-negative rods stand very decent for identification without special extraction procedures [37]. For bloodstream diseases, MALDI-TOF MS is used for microbial pellets collected from positive blood cultures, and nearly 80 percent of pathogens can be easily detected [38]. In about 99 percent of all cases, this recognition is correct. Gram-negative pathogen recognition has been revealed to be extremely useful [23]. The full assessment was conducted once AmpC-producing Enterobacteriaceae were reported, providing their specific trend of antibiotic resistance [39]. Moreover, to differentiate between ESBL-producing and non-ESBL-producing gram-negative bacteria, a new approach based on the MALDI-TOF performance was generated [40]. MALDI-TOF MS shows that ESBL strain identification is feasible and commonly used in clinical microbiology laboratories as a routine test.

A multicenter evaluation of the Bruker MALDI Biotyper Gram-Negative bacteria recognition method was conducted, involving an overall of 2,263 separates containing 23 categories and 61 varieties. The analysis indicated that 99.8 percent of the genus and 98.2 percent of the species were correctly identified by the Bruker MALDI Biotyper method [41]. Also, the detection of anaerobic Gram-negative isolates remained at 91.7 percent to the species

level, and even 92.5 percent of the isolates to the category degree were studied [42]. The MALDI-TOF technique has shown that *Escherichia coli* can be distinguished from the Shigella organisms [43]. Moreover, identify *Enterobacteriaceae* from species of primary culture plates and Carbapenems' development in less than half-hour [44]. Numerous research studies have also been accomplished to test MALD-TOF MS's ability to recognize Nonfermenting Gram-Negative Bacilli, most of which indicate species mostly quarantined from respiratory system secretions of individuals with cystic fibrosis [45-48].

Moreover, MALDI-TOF MS's capability to classify Nonfermenting Gram-Negative Bacilli has been checked for unusual varieties that recognize 29 categories and hit a recognition ratio of 65 percent at species and 28 percent at the level of the genus [37]. After enriching the SARAMISTM database with reference spectra, recognition by MALDI-TOF MS was extended to varieties belonging to the *Acinetobacter calcoaceticus-baumannii* complex to enhance identification efficiency. With the improved database, the MALDI-TOF Vitek MS resulted in a successful alternative to molecular biology methods [49].

3.1.2. Identification of Gram-positive bacteria.

Concerning Gram-positive streptococci, recognizing some gram-positive credits at the specie-level is vital. Since using biochemical or phenotypic approaches in some cases, like the coagulase-negative staphylococci, the viridans cluster streptococci, and even some enterococci may be inaccurate [50]. Compared to traditional phenotypic methods, MALDI-TOF MS can overcome these demerits [51, 52]. Additionally, it also can easily distinguish species of bacteria despite having identical phenotypic features [53]. The VITEK MS method for gram-positive bacteria detection has been evaluated. A total amount of 1146 isolates, representing 13 categories and 42 types, were studied, and also a single accurate assessment of species was given for 93 percent of VITEK MS. In nearly 3 percent of isolates, several potential recognitions were offered, all right at the genus level, whereas in approximately 2 percent of isolates, combined category or inaccurate labels were identified [54]. MALDI-TOF can determine viridans streptococci isolates compared to other approaches that use the sodA mapping techniques as the standard tool studied. One hundred twenty-four clinical isolates were tested from blood samples. MALDI-TOF's sensitivity was 73 percent for the group-level classification and 94 percent for the species-level recognition [55]. These findings present MALDI-TOF as a fast and practical choice for diagnosing streptococci strains of viridans at a group level; however, there are some complications in discriminating related species in some classes very closely.

The efficiency of two MALDI-TOF MS systems, MALDI Biotyper and VITEK MS, was assessed by classifying streptococci group viridans. MALDI Biotyper correctly classified 89 percent and VITEK MS percent of the isolates at the community level, while MALDI Biotyper ranked 75 percent and VITEK MS 97 percent of the analyzed species level. Among the clinical isolates, thirty-six strains were misidentified as Streptococcus pneumoniae by MALDI Biotyper [56]. Thus, this finding depicts results from the research that indicated that the present MALDI-TOF method was a good option for detecting viridans streptococci. Furthermore, to a mass spectra model study, Bruker MALDI Biotyper system was performed with ten reference strains of viridans streptococci in the ClinProTools program to classify 28 clinical isolates of S. Pneumoniae and 47 isolates of *S. mitis*/*oralis*. The findings acquired enhancing the recognition with the ClinProTools were associated with the regular MALDI-TOF title. With the supplemental ClinProTools mass spectra study, the proportions of accurate

detection by the straight transfer and extraction improved to 85.3 percent and 100 percent, respectively, compared with the necessary process [57]. This finding elucidates that after recognizing MALDI Biotyper, ClinProTools' additional mass spectra analysis significantly increased the accuracy of recognition between *S. pneumoniae, S. oralis, S. mitis.* MALDI-TOF MS's efficiency in detecting viridans streptococci within the Anginosus Group was analyzed [58]. Likewise, MALDI-TOF systems' capacity to recognize endocarditis-causing viridans streptococci was evaluated [59]. Hence, these results indicate outstanding results in the Anginosus, Bovis, and Mutants groups, while some disparities were demonstrated for the *Streptococcus mitis, oralis*, or *tigurinus*.

Gram-positive bacilli and actinobacteria, consisting of Mycobacterium species recognition, exemplify the MALDI-TOF function [60]. The implementation of MALDI-TOF MS was assessed for a group of L. monocytogenes isolates. The isolates were cultivated in various development media and evaluated at two incubation periods, 24 and 48 h, respectively. Dependable genus-level identification was accomplished from most media, while the species-level label was affected by culture conditions. Efficient speciation was conducted for chromogenic Agar Listeria Ottaviani Agosti and non-selective horse blood agar cultures after 24 h of incubation [61]. To evaluate Corynebacterium experimental strains' identity in contrast to recognition acquired by Api Coryne and 16S rRNA or rpoB genes sequencing as an indication technique, Bruker Biotyper MALDI-TOF was used. In approximately 89 percent of instances, the alignment between Api Coryne strips and MALDI-TOF-MS recognition was located. MALDI-TOF-MS was incapable of identifying *C. aurimucosum. C. minutissimum* and *C. Singular*, but 95 percent of strains are recognized reliably [62]. A multicenter assessment of MALDI-TOF MS has been used for Gram-positive bacteria assessments to classify 1146 isolates of Gram-positive aerobic bacteria, containing 13 genera and 42 species. 92.8 percent (1063/1.146) proper recognition to the species level and 95.5 percent (1094/1146) to the genus level were shown [54].

MALDI-TOF MS's performance was evaluated to recognize *Mycobacterium* species and subspecies, *Nocardia* species, and other aerobic actinomycetes. The supplied MALDI-TOF MS manufacturer and a customized database developed in the research laboratory compared to the 16S rRNA gene were used [63]. MALDI-TOF MS has become a useful instrument for identifying these groups of microorganisms. However, there have been some weaknesses in the data sources and the capacity to classify gradually developing mycobacteria [64]. Based on these findings, MALDI-TOF-MS is a quick and efficient technique for identifying bacterial types, even though molecular techniques have yet to overcome any limitations.

3.1.3. Identification of anaerobic bacteria.

With biochemical approaches, anaerobes have already been demanding to classify [65]. Assessing the dependability of recognition by MALDI-TOF MS compared to 16S rRNA sequencing of one the most usual scientifically appropriate anaerobic bacteria, namely *Bacteroides spp., Clostridium spp., Prevotella spp., Fusobacterium spp.* and gram-positive anaerobic cocci was studied. Approximately 95 percent of the anaerobes at the category level and 87 percent at the species were determined correctly by MALDI-TOF MS, with recognition errors primarily between non-fragile Bacteroides spp. and the anaerobic gram-positive cocci [66].

3.1.4. Yeast identification.

Yeast infections in immunosuppressed patients are common complications [67]. Remedies for inflammatory conditions, hematologic malignancies, hematopoietic cell transplantation, and even extreme transplantation are linked [8]. Catheter-associated main venous infection, pneumonia, fever, pleuritic chest pain, and hemoptysis resulting from invasive pulmonary aspergillosis is invasive candidiasis [68]. MALDI-TOF MS in regular medical assessments has been advocated because of the time-consuming and high-cost nature of traditional phenotypic techniques applied for yeast recognition. MALDI-TOF MS identified 41 distinct species from 2683 medical yeast isolates. The precise identification of all the yeast isolates was 98.8 percent (2651/2683) [69]. 92.5 percent (185/200) identification rate with 200 scientific yeast isolates was also shown [70]. Furthermore, 98 medical isolates of yeast were evaluated for the recognition rate of MALDI-TOF MS, and outcomes revealed that 74.5 percent (73/98) was determined to species level and 94.9 percent (93/98) to genus level [71]. Hence, MALDI- TOF MS is costly and more straightforward than the existing DNA-based standard gold identification technique.

The level of specificity and sensitivity of yeast classification between research laboratories can be problematic compared to the implemented diverse sample preparation processes [72]. Subsequently, these portions were extraordinarily variable and ranged from 16 percent and 21.3 percent with the straight transfer technique to more than 90 percent after formic acid-ethanol extraction [73, 74]. A hybrid approach based upon the discovery of combined yeast cultures with a chromogenic medium and the colonies' recognition by MALDI-TOF MS to a collection of 15.661 clinical samples, with 6.192 yeasts of 42 different species, was applied. Generally, approximately 99 percent of isolates were determined on the first or second MALDI-TOF MS effort and a consequent average turnaround time of 0.346 days. Combined infections accounted for around 9 percent of the positive samples evaluated. A MALDI-TOF threshold score of 1.8 was considered dependable in the mix with the chromogenic medium subculture to enhance the yeast recognition process and spot combined infection in the clinical research laboratory [75]. This finding illustrates that MS is more comfortable, quicker, easier, and more reliable than biochemical techniques and helps identify closely related organisms. To sustain the increased efficiency of MS in the future, continuous expansion of the library is necessary.

3.1.5. Fungi identification.

The study of colony morphology and microscopic characteristics relies on recognizing molds and filamentous fungi, so identifying filamentous fungi or molds is usually time-consuming and labor-consuming, and even inexperienced laboratories, incorrect outcomes might be generated [76]. The MALDI-TOF spectrum, derived from a fungal isolate, never fully matches the reference database's significant spectrum profile. The clinical mycology laboratory acquired MALDI-TOF MS slowly, especially about molds [71, 77], since the fungal isolate and the hyphal or conidial phenotypes' organic intricacy may exist together in the same isolate has raised some problems [78]. MALDI-TOF MS correctly determined 95.4 percent of isolates for filamentous fungi at the species level [79]. The recognition of medical mold isolates from individuals with aspergillosis, fusariosis, and mucormycosis was examined. The research study developed 2832 reference spectra, representing 347 species of mold, to boost mold recognition. Next, this process recognized 1094 of 1107 (98.8 percent) clinical mold isolates, corresponding

to 107 distinctive species, superior to morphological identification (78.2 percent) [80]. Moreover, in one research using a comprehensive in-house database, MALDI-TOF MS seemed to acquire more precise filamentous fungi recognition [14]. Numerous studies have been carried out to enhance the detection of filamentous fungi and molds [81-83]. These findings indicate that the detection can be improved with sufficient focus on the sample extraction process and by enhancing the number of mass spectra describing each species in the reference collection to compensate for the reasonably low variety of strains readily available to create useful reference spectra collections.

3.1.6. Mycobacterium identification.

Mycobacteria are effective pathogens that pose a severe global public health crisis [84]. *Mycobacterium* is an *Actinobacteria* genus containing more than 190 recognized species. *M. Tuberculosis* is the most morbidity-induced pathogen in humans [85]. Owing to the increasing variety of patients with autoimmune disorders and immunocompromised people, diseases resulting from NTM infection have increased in recent years [86].

The standard for *Mycobacterium* community of bacteria reported in human secretions, like sputum, bronchoalveolar lavage, or pleural fluid, confirmed by smear microscopy, is diagnosed with tuberculosis [87]. However, *Mycobacterium* species' identification is difficult due to its long incubation time for each culture phase and biochemical response [88]. Substance sensitivity is less common than community, such as sputum acid-fast bacilli smear stains [89]. Therefore, *Mycobacterium* detection methods are needed quicker and more precise to boost patient results. Sequencing and probing techniques have recently been used for bacterial detection. Nucleic acid hybridization (NAH) or nucleic acid amplification screening helps identify MTC species in patients with suspected TB.

Comparing MALDI-TOF MS with NAH, MALDI-TOF MS was found to classify 100% of the M. Appropriate strains of tuberculosis; nevertheless, just 38.5% of the NTM strains were typed by NAH [90]. MALDI-TOF MS has been revealed to be remarkably adaptive to the detection of MTC in a diagnostic environment and diminishes the recognition time at lower costs [88]. While MALDI-TOF MS is the most affordable and quickest way to identify MTC, NTM is becoming more clinically relevant. Further research should be done to identify NTM applying MALDI-TOF MS [91]. A technique proposed from the study might diminish the misidentification of *Mycobacterium avium* complex (MAC) by MALDI TOF MS [92]. Some very closely relevant species have analogous spectra, and the MALDI-TOF MS technology cannot distinguish them [93]. *For instance, mycobacterium chimaera and Mycobacterium intracellulare* are also challenging to differentiate from one another.

Nevertheless, *M. intracellulare* is considerably more likely to be linked with a lung infection than *M. chimaera* [94, 95]. The accurate recognition between *M. intracellulare* and *M. chimaera* within MAC may be beneficial in scientific significance [92]. This finding carried out subtype processing by expanding the MALDI spectra study to identify *M. intracellulare* and *M. chimaera* accurately.

It is additionally challenging to differentiate microorganisms within the *Mycobacterium abscessus* complex like *Mycobacterium massiliense* (*M. abscessus subspecies bolletti*) and *M. abscessus* (Sensu Stricto). Various phenotypes of antibiotic resistance are present in the different bacterial subtypes, and their accurate, rapid detection might be valuable for the proper administration of antibiotics. *M. massiliense* can be distinguished explicitly from *M. abscessus* by using genetic algorithms [96]. MALDI Biotyper has an upgraded collection of

mycobacterium, including 164 species. Still, a wide-ranging of NTM data continues to be unreported. The MALDI-TOF recognition efficiency by 157 mycobacterial isolates showed 89.2 percent (140/157) reliable identification for the Mycobacterium species assessment. Additionally, 125 non-tuberculosis mycobacterial (NTM) isolates to assess the MALDI-TOF MS recognition were used [97]. There is 88.8 percent (111/125) of appropriate titles at the species level, with a score of about 1.7 [98]. For 87.3 percent (89/102) and 62.8 percent (64/102) of clinical mycobacterial isolates, MALDI-TOF MS provided accurate identification at genus and species levels from solid culture media, respectively [99].

3.2. Applications in finding drugs.

Not only for physicians but also pharmaceutical firms, increasing the medicine development procedure is a top priority. A high throughput screening analysis is provided by traditional biochemical assays [100]. Nevertheless, they are individual for a single analyte and also usually need labeling reagents [101]. MS has been significantly applied in the biotechnology and pharmaceutical sectors, mainly due to the high sensitivity, speed, and efficiency in carefully identifying biological and chemical compounds [102]. Though MALDI-TOF MS is utterly tolerant of sodium chloride and cleaning agent, affinity capture and SPE-based approaches can be integrated with MALDI to confirm that the samples are cleaned [103]. A novel instrument has been developed using Self-Assembled Monolayers combined with MALDI (SAMDI) to screen and classify small particles' noncovalent binding to specific healthy proteins [104]. SAMDI uses self-assembled monolayers covered with polyethylene glycol and a chemical moiety like biotin to catch protein targets. Samples are evaluated on gold arrays, consistent with the study of MALDI-TOF [105]. Analyte purification lowers the causes of pollution and lessens the impact of ion suppression regardless of the merits of buffer pollutants for the MALDI assay.

From this standpoint, MALDI-TOF MS assays offer a prospective approach for numerous uses, like drug testing and identifying patients expected to react to treatment therapies [106]. A measurable MALDI-MS technique has been created for the therapeutic medication examination of irinotecan, an extensively prescribed antineoplastic agent for treating colorectal cancer [107, 108]. Likewise, to control the remedial levels of immunosuppressant medications in the blood, automated quantitative practices have been established [109]. This checking is especially vital in transplantation after some immunosuppressants' narrow therapeutic intervals [110]. It was feasible to examine amphetamine and methamphetamine in covert tablets in the forensic area using MALDI-TOF MS [111]. In pharmaceutical laboratories, MALDI IMS is implemented as an analytical tool [112]. These findings indicate that this technique, coupled with other approaches, is an essential tool to contribute to the elucidation of processes in developments and improvements in MALDI-TOF strategies, scientific microbiology procedures, and medicinal drug control.

3.3. Microbiological identification of a minimal residual illness.

The efficacy of chemotherapy in hematological conditions like leukemia and multiple myeloma is measured by calculating the so-called minimal residual disease (MRD) [113]. That is also usually focused on examining the monoclonal elements that use nephelometry and immunofixation to circulate in patients' blood [114]. However, this historical technology cannot distinguish between flowing monoclonal immunoglobulins arising from malignancy

and recombinant biopharmaceuticals used mostly for care [115]. Numerous scientific groups have made significant efforts to develop responsive and reliable evaluation standards, which seem to be, above everything, efficient in producing relevant and quickly repeatable findings internationally [116, 117]. Serological values, namely serum-free light chain or immunophenotypic devices on bone marrow or peripheral blood, like multi-parameter flow cytometry and next-generation/high throughput sequencing technologies (NGS), have been generally planned [118-120]. Every immunoglobulin is defined by a distinctive series of amino acids, the impressive mass of which can be applied over time as an indicator for patient disorder monitoring. An accurate estimation of their levels offers prognostic details and a comprehensive summary of the procedure's results [121]. MS methods can reveal monoclonal immunoglobulin in serum; MALDI-TOF MS is the scientific standard tool for studying serum peptides in cases with acute leukemia and biomarkers of peripheral blood detection [122]. These findings imply that this area's applications include assessing prognostic possibility, the medical diagnosis, and evaluating the therapeutic reaction.

3.4. Application for the evaluation of typically sterile fluids pathogenes.

MALDI-TOF has effectively recognized bacteria directly in urine and cerebrospinal fluid, bypassing the need to grow pathogens [123, 124]. Direct indication of microorganisms in CSF by MALDI-TOF MS can offer a fast microbiological medical diagnosis that would allow for primary and adequate care [125]. Very few studies use MALDI-TOF MS to identify bacterial meningitis-causing microorganisms directly. A pneumococcal meningitis case was testified where MALDI-TOF had been fruitfully recognized as the microorganism [126]. MALDI-TOF Vitek MS demonstrated a strange degree of precision for detecting Gram-negative microbes in urine specimens, approximately 105 CFU/mL, aiming to come to be a fast and reliable testing technique for urinary system infection triggered by Gram-negative microbes [127]. These findings suggest that other results on proper pre-treatment are necessary for Gram-positive and yeasts' documentation.

4. Detection of Phenotypical Resistance by MALDI-TOF MS

The detection rate achieved in medical and microbiological research laboratories has resulted in a substantial delay in identifying the pathogen and assessing its resistance and susceptibility to antibiotics [128]. There has also been a demand to establish quick resistance detection or even antibiotic susceptibility tests based on MALDI-TOF MS [129]. For this goal, there are two primary routes. On the one side, to describe drug inactivation through bacterial resistance mechanisms, MALDI-TOF mass spectrometry's capacity to precisely detect mass modifications in small molecules is being used [130]. On the other hand, the strategy is being established to rapidly keep changes in peak patterns and intensities of the microbe spectrum related to drug function [131].

In most cases, resistance to beta-lactam antibiotics is caused by an enzyme's antibiotic degradation [132-134]. The gene encoding this beta-lactamase is often found on a plasmid and can be moved from one strain to the next, raising the probability of spreading it in hospitals [135]. It has been revealed that when the medicine is incubated with resistant microorganisms succeeding a straightforward procedure, MALDI-TOF MS can be expended to classify the cleavage. This approach has been extended to carbapenems [136], but other beta-lactams, such

as ampicillin or cephalosporins, can also detect separation [137]. It was possible to identify beta-lactamase production from microorganisms obtained from positive blood cultures [138].

Likewise, the discovery of the plasmid-mediated quinolone response factor AAC(6')-*Ib-cr* in Enterobacteriaceae has lately been defined [139]. For MALDI Biotyper, an IVD-CE branded prepared testing set was already released that can be applied to identify carbapenemases from enterobacteria, Acinetobacter, and Pseudomonas aeruginosa [91]. The initial MALDI-TOF MS read-out-based diagnostic package and the primary mass spectrometry resistance trial, whose progress reveals the translation from research study to usual diagnostics.

Although these new MALDI-TOF MS tests can diagnose antibiotic resistance and provide insights into specific resistance pathways, they cannot anticipate antibiotics' sensitivity [140]. A shortage of a particular drug inactivation system, in particular, does not preclude other tools that would induce resistance [18]. There is a broad scope for supplementary methods of using MALDI-TOF MS for sensitivity or tolerance analysis. Another approach utilizes the study of healthy protein synthesis happening in cells by supplying them with amino acids classified as isotopes [141]. Adding fatty amino acids boosts the recently synthesized proteins' molecular weight, triggering peak changes in the mass spectral profiles that can also be instantly identified by a software program [142]. By this method, resistance could be detected in about 2 hours. Because the essay needs to be planned as a killing test, applying a high antibiotic concentration, no connection can be established with the MIC used for susceptibility prediction [143].

The latest experiment is very closely similar to classic testing of sensitivity to antibiotics and also, accordingly, can predict actual susceptibility. MBT ASTRA (MALDI Biotyper Antibiotic Susceptibility Test-Rapid Assay) approximates a microorganism's development or even no development following short incubation [144]. Mass spectra are attained following the microorganism's incubation with antibiotics and without antibiotics [145]. After calculating MALDI-TOF MS, the microorganism's relative development is measured by comparing the microorganism's unique peaks to an interior norm [146]. Although the antibiotic subdues a susceptible organism's result, an immune one can increase and provide a range of high-intensity MALDI-TOF profiles. Some types have already been successfully screened for prescription antibiotics in various groups [145]. The test's successful application to microorganisms straight collected from positive blood cultures was demonstrated [147]. Thus, the ASTRA assay is a successful prospect in clinical microbiology for a widely appropriate antibiotic susceptibility examination [148]. The biggest obstacle to translating the assay into a regular test seemed to be the process of centrifugation, which is not aligned with a standard high throughput process in a bacteriological laboratory [53]. A novel assay was documented to remove centrifugation steps by increasing microorganisms straight at the MALDI target [149, 150]. Since this examination requires relatively longer incubation times, implementing MALDI-TOF MS as a common AST technique may be a necessary development step.

5. Strategy of Subtyping by MALDI-TOF MS Peptide/Protein Profile

Identifying conventional biochemical microorganisms offers only the species' outcomes [151]. MALDI-TOF MS can be implemented to attain the protein profile of the strain [152]. There is advantageous to uncovering phenotype-related biomarkers using the protein profile, like drug-resistant strains or analogous species classification [153]. The Subtyping technique uses the protein profile to acquire the subtype information using the same protein

profile following the strain recognition data [154]. It can also be used to identify drug-resistant strains or classify microbial species complexes.

Preclinical findings of antibiotics-resistance strains may be linked with methicillin-resistant *S. aureus* (MRSA) pieces [155]. Loads of the cfiA positive *B. fragilis* strains are carbapenem immune [156]. A bla_{KPC} positive linked signal can be significantly associated with *K. pneumoniae* carbapenem resistance strain, producing *K. pneumoniae* carbapenemase (KPC) [152]. Reliable recognition of a microbial species complex might be applied for the *Listeria monocytogenes* cluster (consisting of *L. monocytogenes, Listeria ivanovii, Listeria innocua, Listeria welshimeri, Listeria seeligeri*, as well as *Listeria grayi*) demonstrate just minor variation in genetic and proteomic profile stages [149].

6. Limits of microorganisms' recognition by MALDI-TOF MS

MALDI-TOF MS is not appropriate for distinguishing between *Shigella* and *E. coli*, *Bordetella pertussis, B. bronchioseptica, Achromobacter xylosoxidans,* and *A. ruhlandii*, as well as *Bacteroides nordii* and *B. salyersiae* [154]. The Enterobacter cloacae complex comprises six extremely related varieties (*E. asburiae, E. cloacae, E. hormaechei, E. kobei, E. ludwigii*, and *E. nimipressuralis*) with matching resistance patterns that are still not distinguished [151]. Furthermore, pneumococci, viridans streptococci, and encapsulated bacteria, like *K. pneumoniae* and *H. influenza*, can be incorrectly identified by MALDI-TOF MS [145]. Through the use of reference spectra, several of these bacterial species can be classified. Nevertheless, a handful of these strains with an extremely comparable hereditary history or a wide variety of inter-species cannot be identified by the mass spectrometry signal and attain high-confidence recognition results [156].

Moreover, at present, accurate NTM detection is not practical. The reference spectrum can restrict the significant limitation of MALDI TOF MS classification for filamentous fungi. Continual improvements of spectral databases and optimum model upgrading within the MALDI-TOF MS system will surge its power and possible tasks.

7. Conclusions

MALDI-TOF MS is a modern technology that has recently utilized microorganism recognition, a cost-effective, high-throughput system with high precision. It is particularly pivotal for regular medical microbiology. The findings might come from positive BCs, urine, other biological fluids, agar plates, and broth media subcultures. Several studies have confirmed the reliability and precision of MALDI-TOF MS, and the accuracy of microorganism detection is critically based on the total records in the database. Hence, constant upgrading of the reference database is imperative for developing results. Additional database improvements in the MALDI-TOF MS system enable the rapid identification of antibiotic resistance features and integrity and the direct identification of pathogens in urine samples. In the future, this approach will have substantial capacity in analysis research laboratories.

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Conflicts of Interest

The authors declare no conflict of interest.

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