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Review: Identification and diagnosis of hospital-important microorganisms using MALDITOF and metagenomic NGS

Revisión: Identificación y diagnóstico de microorganismos de importancia hospitalaria mediante MALDITOF y NGS metagenómica

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ABSTRACT. The identification and diagnosis of pathogenic microorganisms are important for the treatment, management, and prognosis of hospital infections. However, many microorganisms are difficult to identify using conventional diagnostic methods. Advanced techniques such as MALDI-TOF MS and mNGS offer advantages for identifying microorganisms of hospital importance. MALDI-TOF MS is a rapid and precise method that creates a mass spectral fingerprint for microbial identification down to strain levels. This technique is quick, sensitive, and economical, allowing the identification of different types of microorganisms. MALDI-TOF MS can only identify new microorganisms if the spectral database contains peptide mass fingerprints of specific strains, which is a limitation of the technique. Meanwhile, metagenomics allows the analysis of DNA segments from multiple microorganisms within a community, either through amplicon-based or shotgun sequencing. Advances in clinical next-generation metagenomic sequencing (mNGS) increase diagnostic capacity by rapidly detecting rare pathogens and antibiotic resistance genes. This technique presents some limitations in its accuracy and logistical complexity but can perform comprehensive analyses of microbial communities. Both methods complement traditional diagnostic techniques, with MALDI-TOF MS being preferred for rapid identification of known pathogens from pure cultures, while metagenomic NGS is recommended for detecting uncultivable or rare microorganisms in complex samples. This review summarizes MALDI-TOF MS and metagenomics as alternative methods for identifying and diagnosing microorganisms in hospitals. In conclusion, this review provides a comprehensive comparison of MALDI-TOF MS and metagenomic NGS, emphasizing their complementary roles in clinical microbiology and offering practical insights into their application in hospital settings, which distinguishes it from previous reviews focused solely on individual techniques.

Keywords. *DNA sequencing, mass spectrometry, metagenomics, pathogen identification, microbial communities, microbiological diagnosis, ribosomal proteins.*

RESUMEN. La identificación y diagnóstico de microorganismos patógenos resultan críticos para el tratamiento, manejo y pronóstico de las infecciones hospitalarias. Sin embargo, los métodos diagnósticos convencionales frecuentemente fallan en identificar numerosos patógenos. Técnicas avanzadas como MALDI-TOF MS y mNGS (secuenciación de nueva generación metagenómica) ofrecen ventajas significativas para identificar microorganismos de relevancia hospitalaria. MALDI-TOF MS constituye un método rápido y preciso que genera un perfil de espectrometría de masas para la identificación microbiana hasta niveles de cepa. Esta técnica resulta rápida, sensible y económica, permitiendo la identificación de diversos microorganismos. Su limitación principal radica en que solo puede identificar nuevos microorganismos si su base de datos espectral contiene los perfiles de masas peptídicas de cepas específicas. La metagenómica permite el análisis de segmentos de ADN de múltiples microorganismos dentro de una comunidad. Los avances en mNGS clínico amplían la capacidad diagnóstica al detectar rápidamente patógenos raros, no cultivables y genes de resistencia antibiótica. Esta técnica presenta limitaciones en precisión y complejidad logística, pero posibilita análisis integrales de comunidades microbianas. Ambos métodos complementan a las técnicas tradicionales. MALDI-TOF MS se prefiere para la identificación rápida de patógenos conocidos desde cultivos puros, mientras que mNGS se recomienda para detectar

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microorganismos no cultivables o raros en muestras complejas. Esta revisión resume MALDI-TOF MS y metagenómica como métodos alternativos, proporcionando una comparación exhaustiva que enfatiza sus roles complementarios en microbiología clínica y ofrece perspectivas prácticas para su aplicación en entornos hospitalarios, distinguiéndose así de revisiones previas centradas únicamente en técnicas individuales.

Palabras Clave. *Secuenciación de ADN, espectrometría de masas, metagenómica, identificación de patógenos, comunidades microbianas, diagnóstico microbiológico, proteínas ribosomales.*

1. Introduction

Microorganisms are diverse unicellular beings in various forms and sizes, observable under a microscope, and present in all environmental matrices: water, air, and soil. Some can cause infections or diseases in living beings and are known as pathogens, while others can be considered beneficial.

In the hospital environment, we can encounter different infections caused by microorganisms, and it has been observed that the biological composition of that indoor environment (air matrix) can be determined by the occupants and correlated with the solid surfaces present in it [1] Therefore, the timely and accurate identification of pathogens is crucial for the proper diagnosis and treatment of diseases [2].

The methods for identifying microorganisms can be divided into two groups: phenotypic and genotypic. The phenotype refers to the observable characteristics of an organism, such as morphology, nutritional requirements, or behavior in response to a molecule, while the genotype is the complete hereditary information of an organism encoded in its DNA [3].

Currently, culture-based techniques and biochemical tests are the gold standard in the clinical laboratory for microbial identification. However, increasingly, techniques such as sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) are being used for the same purpose.

Therefore, in this review article, the advantages and limitations of two analytical techniques of great utility in the identification and diagnosis of hospital-relevant microorganisms are compared: MALDI-TOF and metagenomics.

MALDI-TOF is a mass spectrometry technique that uses matrix-assisted laser desorption/ionization time-of-flight, allowing the identification of microorganisms from pure cultures by analyzing ribosomal proteins, separating, and detecting ions in the gas phase. The primary components of its system are an ionization source, a mass analyzer, and a detector. The result of applying an ionization source to a molecule is the formation of ions generated by the excess or loss of electrons. Samples are coated in an organic matrix, which crystallizes upon contact with air, and is then irradiated by a laser. The laser energy causes the crystallized matrix to break apart, generating a cloud of particles. The ions from this cloud are extracted by an electric field. The obtained ions are directed to the mass analyzer and then to the detector [4].

1.1. Precision and Specificity of MALDI-TOF

The MALDI-TOF technique is considered a rapid, accurate, and cost-effective method for microbial characterization and identification. This technology generates characteristic mass spectral fingerprints that are unique signatures for each microorganism, making it ideal for precise microbial identification at the genus and species levels, with the potential to be used for strain typing and identification [5]. MALDI-TOF MS has been used to characterize a wide variety of microorganisms, including bacteria, fungi, and viruses [6], offering many advantages such as rapid identification of microorganisms, allowing its use in various areas including clinical diagnostics. Its accuracy, even at the species level, is over 90% [7]. However, one of its limitations is the need to update the database to improve identifications with the instrument [8],[9]. The accuracy of identification using this technique depends on this database.

1.2. Analysis of Organic Molecules

MALDI-TOF is also a technique used to analyze organic molecules such as nucleic acids, solutions with organic molecules, proteins, and microbial cells. It is a

soft ionization technique that allows the ionization and vaporization of large non-volatile biomolecules, such as intact proteins [10] , which generate single-charge ions, and the spectra derived from MALDI can include more proteins. However, proteins and whole microbes are mostly used in microbiology applications [11], with ribosomal proteins being the most reliable and commonly used biomarkers for bacterial identification.

Another advantage of the MALDI-TOF technique is linked to the two existing sample preparation methods: direct transfer and protein extraction [12]. With MALDI-TOF, intact microorganisms can be processed directly without prior treatment, as most vegetative bacteria lyse after exposure to water, organic solvent, and/or strong acid in the MALDI matrix. When MALDI needs to analyze resistant microorganisms, such as some viruses, bacterial spores, and yeast cells, strong organic acids and/or alcohols are typically added in the pretreatment stages. Similarly, for some bacterial species (such as Actinomyces), specific pretreatment or protein extraction procedures can be useful [13].

The second method to compare is metagenomic sequencing, which is considered by many authors as the "microbiology of the future" because it allows obtaining genome sequences of different microorganisms without the need to culture them. This is of great importance, as there is much information with potential biotechnological applications within the non-culturable portion of microorganisms. For these reasons, metagenomics represents an opportunity to broadly elucidate the biotechnological potential embedded in microbial communities [14].

Metagenomics is based on gene cloning, and it is important to consider the type of sample to select the ideal extraction method. With Next Generation Sequencing (NGS) technologies, millions of DNA molecules can be sequenced simultaneously, greatly facilitating the study of microbial diversity. Its basic procedure involves a series of steps:

First, all genes from the sample are obtained and enriched [7]: 1) segmentation of DNA into several fragments, 2) labeling of DNA using primers or adapters that indicate the starting point for replication, 3) amplification of DNA fragments through polymerase chain reaction (PCR). Second, these genes are cloned into a vector that transforms into host bacteria for the construction of the metagenomic library: 4) sequencing or reading of DNA fragments. The metagenomic library analyzes: 5) reconstruction of the complete sequence using reference sequences and exportation to data storage files [15]. Within this process, DNA extraction and screening of the metagenomic library are highly important.

When sequencing bacterial communities, two metagenomic sequencing methods can be used: amplicon-based metagenomics and shotgun metagenomics.

1.3. Amplicon-Based Sequencing

Short-read sequencing based on amplicons of marker genes, particularly the 16S rRNA gene (16S) and its variable regions (V1-V9), is widely used in exploratory ecological research. Universal marker genes include bacterial rpoB or fungal internal transcribed spacer 1/2 (ITS-1/2). 16S gene sequencing has been applied in the diagnosis of urinary tract infections, abscesses, and sepsis [70]. Similarly, the microbiome of intensive care units (ICUs) showed greater microbial diversity than culture-based techniques [16].

1.4. Shotgun Metagenomic Sequencing

Shotgun metagenomic sequencing allows for unbiased sequencing of microbial genomes in a sample. The data generated by this technique are more complex compared to amplicon-based sequencing. Certain studies have demonstrated the utility of this technique in identifying pathogens in culture-negative conditions, such as pneumonia, meningitis, encephalitis, and sepsis [17]. The sensitivity of this technique is affected by slow microbial growth or low pathogen abundance, leading to the development of different protocols to enhance sensitivity. Validation and comparative studies have been conducted to assess the performance of shotgun metagenomics versus 16S rRNA amplicon sequencing in pathogen identification.

Due to recent advances in DNA and RNA sequencing techniques, metagenomics holds great promise in clinical diagnostics. Although this technique presents logistical challenges for implementation in hospital settings, it provides a faster generation of comprehensive and clinically relevant data [18]. High-fidelity data analysis enables predictions of new taxa and the reconstruction of genomes from organisms that cannot be cultured in vitro [19].

By not relying on traditional cultivation methods, next generation metagenomic sequencing offers unbiased sampling, which is beneficial in detecting rare or novel microorganisms and enhances analytical sensitivity for identifying fastidious microorganisms and diagnosing pulmonary coinfections [20].

2. Methodology

Several search platforms were used for the random location of bibliographic documents, including Google Scholar, paperpile, Scopus, Google search engine, and PubMed. Searches were performed using keywords such as MALDI-TOF, identification techniques, metagenomics, MALDI-TOF vs. metagenomics, cell cultures, clinical metagenomics, microbiological diagnosis, molecular detection. The retrieved records were systematically classified into books and scientific research articles, with approximately 49 articles considered for review. The reviewed articles were produced between 2010 and 2024.

For the selection of useful articles for the analysis of this review, specific inclusion and exclusion criteria were applied. Articles published in peer-reviewed journals, written in English or Spanish, and that directly addressed the use of MALDI-TOF MS and/or metagenomic sequencing for the identification and diagnosis of microorganisms relevant to the hospital were included. Additionally, preference was given to articles published in high-impact journals that provided significant advances or comprehensive reviews on the topic. The quality of the selected references was evaluated based on their publication in peer-reviewed journals, impact factor, and relevance to clinical microbiology. Preference was given to recent studies (2018-2024) and published in high-impact journals. Articles that were not related to clinical microbiology, focused solely on non-pathogenic microorganisms, or lacked sufficient methodological detail were excluded (Table 2).

In this way, the main characteristics or comparisons considered for the chosen techniques were evaluated. This included advantages and limitations of the technique, physical principles, chemical foundations, fundamental components, applications in the hospital sector, among others.

3. Development and Discussion

3.1. Metagenomics

Conventional methods used in clinical microbiology allow the identification and diagnosis of many pathogens causing hospital-acquired infections. However, there are concerns about their ability to detect multiple pathogens, non-culturable microorganisms or those present in low concentrations. An alternative method employed in recent years is next-generation sequencing (NGS) or high-throughput sequencing [17], [21]. As a result of these new technologies, next-generation metagenomics (mNGS) is increasingly used in bacteriology and infection diagnosis [22], [23], [24]. Furthermore, this technique is employed to predict antibiotic-resistant phenotypes [19], identify pathogen virulence genes and the understanding of antibiotic resistance and virulence factors [25].

Recent studies have demonstrated the higher sensitivity and specificity of NGS metagenomics in diagnosing hospital-acquired infections compared to traditional culture methods [23], [26], [27]. It promotes rapid detection of pathogenic bacteria [18], [28] and infections caused by rare organisms [29], [30], and it can also detect fungi [31], [32] and viruses [7]. NGS sequencing is useful for adjusting antibiotic treatment regimens [24], [33], [34]. However, mNGS has limitations in terms of its clinical application [17], [22], [24], [30], [35]. mNGS is a short-read sequencing method, so the read length limits the efficiency of turnaround time; fragment lengths range from 50 to 300 base pairs; it is unable to detect flow errors before completing the process; it is non-portable and requires a fixed infrastructure with maintenance resources, making genomic characterization limited and costly. It requires support from conventional methods [30], [32], [36]. Additionally, validating its results requires other techniques, which adds to cost and time. DNA sequencing without simultaneous RNA sequencing can lead to false-negative results for certain viruses [35]. Due to its high cost, it is not cost-effective for routine diagnosis of mild or moderate infections; it is technically complex, and there are no guidelines available to assist with result interpretation. mNGS has lower specificity compared to conventional molecular techniques, which can lead to incorrect diagnoses and treatments in clinical settings [31]. Another limitation of mNGS is its technical complexity, as well as the analysis of its results, which

require thorough bioinformatic and clinical processes for proper interpretation; furthermore, there are no guidelines available to assist with result interpretation [31], [37]. mNGS has lower specificity compared to conventional molecular techniques, which can lead to incorrect diagnoses and treatments in clinical settings [29]. This limitation is supported by recent studies, who highlight that the interpretation of mNGS data can be challenging due to the presence of background noise and non-pathogenic microbial sequences, which may reduce its specificity in clinical applications [28].

Metagenomic analysis has a broad spectrum of detection for pathogens present in the sample without relying on culture. It also allows for the assembly of complete or near-complete genomes of microorganisms from such samples [38][50]. The technique can enhance diagnostic efficacy when complemented with other techniques, such as traditional culture and biochemical assays [24]; description of phenotypic characteristics and proteomic information obtained by MALDI-TOF MS [22]; RNA/cDNA-targeted sequencing [35] and metatranscriptomic assays [24]; its efficacy in the final clinical protocol will depend on differences between studies, including patient populations, study designs, and definitions used to determine clinical impact [31].

For example, in cases of culture-negative sepsis, protocols such as host DNA depletion and targeted enrichment have been shown to significantly improve the sensitivity of metagenomic NGS, as demonstrated by Li et al (2023) in their study on bloodstream infections [33].

3.2. MALDITOF-MS

Molecular and genomic techniques take 3 to 5 days for pathogen identification and studying antibiotic resistance, in addition to requiring trained laboratory personnel. This is not practical for clinical diagnosis [39].

To solve this problem, the technique of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used as an alternative [40], [41], [42]. This technique is faster (takes only 24 hours) [4], [43], has lower cost [41], [44], [45], allows for labor savings, and has greater validity in the clinical microbiology laboratory process [4]. MALDI-TOF enables the identification of various types of microorganisms from cultures, or also to work directly with biological samples [9], [41]. It is highly sensitive for species-level identification [4]. It has its own library of reference spectra, which is commercialized and possible to expand the available databases along with the equipment [41]. MALDI-TOF MS systems, in addition to speeding up microbial identification, are very useful for identifying microorganisms that are difficult to identify with traditional methods [46], [47]. However, Abd El-Aziz considers that: "Metagenomic qPCR could identify a wide range of bacteria directly from blood and pus with more sensitivity, greater discriminatory power, and a shorter response time than those using conventional culture. This could allow for timely administration of rapid treatment" [48]. Calderaro and Chezzi affirm that: "There are some limitations in the use of MALDI-TOF MS in the clinical microbiology laboratory and they require further development and more advanced technology" [4]. The cost of the device remains a limitation for its introduction; in addition, it is necessary to subculture microbial species, because the biomass must be composed of between 102 and 104 cells; fresh culture is required along with pretreatment for the identification of fungi/molds and mycobacteria. For these reasons, this technique entails higher cost, additional time, and trained personnel in the laboratory to achieve microbial identification, and it does not allow for the elimination of conventional assays to identify these microorganisms. Furthermore, the use of pretreatment protocols to improve these limitations has not been sufficiently investigated. Commercial devices available for MALDI-TOF MS cannot identify and differentiate mixed cultures of microorganisms, in vitro cultures, or apply directly to biological samples. It is also not possible to discriminate microorganisms isolated in pure cultures that belong to biologically related species, and additional tests are required for complementation [4], [49].

The general characteristics of sequencing technique and MALDI-TOF MS technique are summarized in Table 1.

4. Conclusions

This review highlights significant advances, as well as applications of two emerging methodologies in the identification and diagnosis of clinically important microorganisms. Metagenomic analyses provide a broad spectrum of detection that allows for the identification of microorganisms present in a sample without the use of culturing methods. With genomic techniques, complete or nearly complete genomes of samples can be assembled, and genomic sequencing data can be used for various bioinformatic analyses.

Challenges of metagenomic techniques include the complexity of sample preparation, the need for high-quality DNA extraction, extensive bioinformatic analyses required to interpret the large amount of sequencing data, the high cost associated with computational equipment, sample sequencing, and trained personnel, which can limit access to certain institutions.

MALDI-TOF technique offers a rapid and accurate identification of bacteria, has a lower cost per sample than metagenomic techniques, and does not require highly trained personnel for its handling or data analysis. Recent studies have confirmed that MALDI-TOF MS achieves an accuracy of over 90% for species-level identification, particularly when using updated spectral databases [4]. The cell wall structures of microorganisms are a limitation in identification with the MALDI-TOF method, and an updated and comprehensive database is required for precise species-level identification.

Table 1. Characteristics of MALDI-TOF MS and mNGS.

Characteristic	NGS (Next Generation Sequencing)	MALDI-TOF
Operating Principle	DNA/RNA Sequencing	Protein mass spectrometry
Sample Preparation	Extraction of high-quality nucleic acids, library preparation, and sometimes amplification	Minimal, often direct lysis in matrix solution
Type of Data Generated	Genetic sequences	Protein mass spectra
Identification Capabilities	Wide range, including uncultivable and new species	Known organisms, dependent on database
Speed of Results	Hours to days	Minutes to hours
Cost	High	Low after initial investment
Processing Capacity	High, capable of processing multiple samples in parallel	High for protein analysis, one sample at a time
Data Analysis	Complex, requires resources and expertise in bioinformatics	Simpler, matching of spectra with database
Accuracy and Sensitivity	High accuracy, detects low abundance sequences	High accuracy for known organisms
Database Dependency	Less dependent, can discover new sequences	Highly dependent on the quality and size of the database
Applications	Microbial profiling, resistance gene detection, metagenomics, epidemiological studies	Rapid identification of bacteria, fungi, and other microorganisms in clinical settings
Limitations	High cost, complex sample preparation, need for extensive data analysis	Limited by the database, issues with some organisms, less effective for uncultivable species

While NGS is less dependent on existing databases, reference databases remain crucial for accurate pathogen identification and result interpretation. This is because comparison with known sequences allows for more reliable taxonomic assignment and reduces the likelihood of false positives or misidentifications.

Genomic techniques have the potential to improve the identification and diagnosis of clinically relevant microorganisms. In resource-limited settings, there is a need for ongoing investment in technology and training of personnel to fully utilize their benefits. Future research should focus on overcoming current limitations, reducing costs, and complementing traditional methods with these more advanced technologies to improve diagnostic accuracy and better address diseases in hospital settings.

Despite their advantages, the implementation of these advanced techniques in hospital settings presents significant logistical and economic challenges. The high initial investment in equipment, the need for continuous training of personnel, and the complexity of integrating these technologies into existing clinical workflows are critical barriers. Additionally, a thorough cost-benefit analysis is essential to justify their adoption, especially in resource-limited settings. Future efforts should focus on reducing costs, improving accessibility, and developing standardized protocols to facilitate the widespread use of these technologies in clinical microbiology.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

CONTRIBUTION AND APPROVAL OF THE AUTHORS

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JV, Literature Review, Conclusion, Summary. 20%

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Annex 1

Table 2. Inclusion and exclusion criteria for reviewed articles and quality assessment of source

#	Reference	Year	Focus	Type of Study	Reliability
1	Cruz-López et al.	2023	Hospital microbiota and healthcare-associated infections	Review	High: solid methodology, experimental support and publication in a high-impact journal.
2	Yang et al.	2024	Comparison of metagenomic NGS and conventional culture for diagnosing infections	Comparative Study	High: prospective design, robust comparative methodology and publication in a recognized scientific journal.
3	Castro F.	2020	Microbial culture collections	Methodological Review	High: Based on recognized institutional practices, detailed approach and the authority of its authors in the field of microbiology and microbial conservation.
4	Calderaro & Chezzi	2024	MALDI-TOF MS in clinical microbiology laboratories	Review	High: solid methodology, reproducible data and publication in a high-impact journal in the scientific field.
5	Torres-Sangiao et al.	2021	Applications of MALDI-TOF MS in clinical microbiology	Review	High: solid methodology, reproducible data and publication in a high-impact journal in the scientific field.
6	Giebel et al.	2010	Microbial fingerprinting using MALDI-TOF MS	Research Article	High: detailed technical approach, support in experimental data and publication in a recognized scientific series in the field of applied microbiology.
7	Yu et al.	2024	Comparison of metagenomic NGS and blood culture for bloodstream infections	Comparative Study	High: robust methodological design, reproducible data and publication in a high-impact journal in the scientific field.
8	Altun et al.	2015	Rapid identification of bacteria from blood cultures using MALDI-TOF MS	Research Article	High: prospective design, use of advanced methodologies such as MALDI-TOF MS and publication in a prestigious scientific journal.
9	De Carolis et al.	2014	Application of MALDI-TOF MS in clinical diagnostic microbiology	Review	High: comprehensive and critical approach, backed by previous data and studies, and publication in a recognized scientific journal in the field of clinical microbiology.
10	Emonet et al.	2010	Mass spectrometry methods in clinical microbiology	Review	High: comprehensive and critical approach, backed by previous data and studies, and publication in a recognized scientific journal in the field of clinical microbiology.
11	Dekker & Branda	2011	MALDI-TOF MS in clinical microbiology laboratories	Review	High: comprehensive and critical approach, backed by previous data and studies, and publication in a recognized scientific journal in the field of clinical microbiology.
12	Singhal et al.	2015	MALDI-TOF MS for microbial identification and diagnosis	Review	High: comprehensive and critical approach, backed by previous data and studies, and publication in a recognized scientific journal in the field of clinical microbiology.
13	Bizzini et al.	2011	MALDI-TOF MS for identifying difficult-to-identify bacterial strains	Research Article	High: solid methodological design, exhaustive data analysis and publication in a recognized scientific journal in the field of clinical microbiology.

14	Quince et al.	2017	Shotgun metagenomics from sampling to analysis	Review	High: Comprehensive approach, backed by previous data and studies, and publication in a renowned scientific journal in biotechnology.
15	Rubio et al.	2020	Next-generation sequencing (NGS) in clinical practice	Review	Medium reliability: provides valuable information on NGS in clinical practice, but it is a review that summarizes existing knowledge and does not present original or experimental data of its own.
16	Oberauner et al.	2015	Bacterial communities in clean rooms and intensive care units	Research Article	High: detailed approach, support in previous data and studies, and publication in a recognized reference work in the field of metagenomics.
17	Li et al.	2021	High-throughput metagenomics for pathogen identification in clinical settings	Review	High: Comprehensive approach, backed by data and previous studies, and publication in a scientific journal recognized in the field of innovative methodologies.
18	Hasan et al.	2020	Metagenomics-based diagnostic approach for central nervous system infections	Research Article	High: robust methodological design, use of advanced technology and publication in a recognized scientific journal.
19	De Abreu et al.	2021	Metagenomic approaches to analyze antimicrobial resistance	Review	High: Comprehensive approach, backed by data and previous publications, and publication in a recognized scientific journal in the field of genetics and molecular biology.
20	Diao et al.	2022	Metagenomic NGS for diagnosing lower respiratory tract infections	Review	High: Comprehensive approach, backed by data and previous publications, and publication in a recognized scientific journal in the field of biomedical research.
21	Ashfaq et al.	2022	Application of MALDI-TOF MS for identification of environmental bacteria	Review	High: Comprehensive approach, backed by data and previous publications, and publication in a recognized scientific journal in the field of environmental management.
22	Ben Khedher et al.	2022	Challenges of 3rd generation sequencing for clinical bacterial studies	Review	Medium Reliability: scientific approach and is based on relevant sources, well founded, although it is necessary to combine it with additional experimental studies for a more solid validation.
23	Cao et al.	2024	Diagnostic value of NGS in infectious diseases	Umbrella Review	Medium Reliability: Although the review provides valuable information on the potential of NGS in the diagnosis of infectious diseases, the presence of biases in the analyzed studies limits the robustness of the conclusions.
24	Zhao et al.	2021	Targeting RNA with NGS for pathogen identification in clinical samples	Research Article	High: robust experimental design, use of advanced technologies and publication in a reputable scientific journal.
25	Hilt, E. E., & Ferrieri, P.	2022	reviews the impact of next-generation sequencing technologies on microbiological diagnostics, including pathogen identification, antibiotic resistance and virulence genes.	Review	High: peer-reviewed article on next-generation sequencing technologies in diagnostic microbiology, published in an indexed scientific journal.
26	Duan et al.	2021	Diagnostic value of metagenomic NGS in infectious diseases	Review	High: It was published in a well-established open access journal, and presents a systematic analysis of the value of metagenomic sequencing in the diagnosis of infectious diseases.

27	Chen et al.	2023	Metagenomic NGS for diagnosing Pneumocystis jirovecii pneumonia in pediatric patients	Research Article	High: published in a renowned journal in the field of clinical microbiology and antimicrobials. The study has a specific focus on the diagnosis of a critical lung infection in a vulnerable group, making it relevant and valuable in the current clinical context.
28	Li et al.	2022	Metagenomic NGS for microbiological diagnosis of abdominal sepsis	Research Article	High: Published in Frontiers in Microbiology, a renowned peer-reviewed journal in the field of microbiology. It addresses an important clinical problem and presents mNGS as a potential tool to improve diagnostics in critically ill patients, making it highly relevant in the current medical context.
29	To et al.	2019	Evaluation of MALDI-TOF MS for identifying Group B Streptococcus	Research Article	High: published in BMC Research Notes (peer-reviewed) covering scientific research in all areas of biomedical sciences. The study focuses on an important assessment of the improvement of bacterial diagnostic techniques, a relevant and useful topic in the clinical setting.
30	Song et al.	2024	Clinical value of mNGS in bronchoalveolar lavage fluid for severe pneumonia	Randomized Clinical Trial Protocol	Medium reliability: Since this is a study protocol yet to be conducted, the reliability of this article would be classified as medium.
31	Pang et al.	2024	Evaluation of plasma microbial cell-free DNA sequencing for predicting infections	Multicenter Retrospective Study	High: Peer-reviewed journal, retrospective multicenter study, robust methodology.
32	Shen et al.	2023	Metagenomic NGS for diagnosing pediatric pneumonia through bronchoalveolar lavage fluid	Cohort Study	High: peer-reviewed journal, large real-world cohort study, robust clinical application.
33	Li et al.	2023	Clinical application of metagenomic NGS in sepsis of immunocompromised patients	Research Article	High: Peer-reviewed journal, specific clinical application in immunocompromised patients, use of mNGS in sepsis.
34	Mao et al.	2024	Utility of paired plasma and drainage fluid mNGS in diagnosing acute intra-abdominal infections	Research Article	High: Peer-reviewed journal, clinical study on the use of mNGS in intra-abdominal infections with sepsis, robust methodology.
35	Chien et al.	2022	Utility of metagenomic NGS for etiological diagnosis of sepsis in ICU patients	Research Article	High: Peer-reviewed journal, clinical study on mNGS in sepsis in ICU, robust methodology and relevant to clinical practice.
36	Lv et al.	2023	Clinical values of metagenomic NGS in patients with severe pneumonia	Systematic Review and Meta-analysis	High: systematic review and meta-analysis in peer-reviewed journal, focus on mNGS for severe pneumonia, strong methodological support.
37	Siller-Ruiz et al.	2017	Rapid identification methods for bacteria and fungi using MALDI-TOF MS	Review	High: review on rapid microbiological identification methods in a specialized journal, with focus on MALDI-TOF and chromogenic media.
38	Thoendel et al.	2018	Identification of prosthetic joint infection pathogens using shotgun metagenomics	Research Article	High: Peer-reviewed journal, shotgun metagenomics study in prosthetic joint infections, robust clinical application.

39	Surányi et al.	2023	Comparing MALDI-TOF MS and sequencing-based identification techniques for microbial monitoring	Research Article	High: Peer-reviewed journal, comparison of microbial identification techniques with MALDI-TOF MS, Sanger and NGS, applicability in environmental monitoring.
40	Alizadeh et al.	2021	MALDI-TOF MS applications in clinical microbiology	Review	High: Peer-reviewed journal, review on applications of MALDI-TOF in clinical microbiology, addresses a well-established technology in clinical diagnostics.
41	Conza	2022	Applications of MALDI-TOF MS in clinical microbiology	Review	High: Peer-reviewed journal, review on the use of MALDI-TOF in clinical microbiology, relevant and well-established topic.
42	Oviaño & Rodríguez-Sánchez	2021	MALDI-TOF MS in the 21st-century clinical microbiology laboratory	Review	High: Peer-reviewed journal, comprehensive review on the use of MALDI-TOF in modern clinical microbiology, well-established topic.
43	Patel	2015	MALDI-TOF MS for the diagnosis of infectious diseases	Review	High: high impact journal, review article on the application of MALDI-TOF MS in microbiological diagnosis, well-founded and widely accepted topic.
44	Cordovana et al.	2019	MALDI-TOF bacterial subtyping to detect antibiotic resistance	Research Article	High: specialized journal in clinical mass spectrometry, focused on a relevant and applied topic of antibiotic resistance, with recognized authorship in the area.
45	Ghurye et al.	2016	Metagenomic assembly: Overview, challenges, and applications	Review	High: publication in a high-impact journal, technical and in-depth focus on metagenomic assembly, applicable in computational biology and metagenomics.
46	Litterio et al.	2024	Comparison of two MALDI-TOF MS systems for identifying anaerobic bacteria	Research Article	High: recent publication in a specialized scientific journal, comparison of anaerobic bacteria identification systems, relevant to clinical microbiology.
47	Moreno et al.	2019	First isolation of Candida auris in Chile	Case Report	Media: Article about a specific case of Candida auris in Chile, relevant to infections, but older publication and in a national journal.
48	Abd El-Aziz et al.	2021	Real-time PCR vs. MALDI-TOF MS and culture-based techniques for diagnosing infections	Comparative Study	High: Comparative study of modern diagnostic techniques in infections, recently published in a high-impact scientific journal.
49	Rychert	2019	Benefits and limitations of MALDI-TOF MS for microbial identification	Review	media: review article on MALDI-TOF, with a focus on its benefits and limitations, although not based on primary studies.
50	S. Purushothaman et al.	2022	Combination of WGS and metagenomics for microbiological diagnostics	Review	High: Comprehensive synthesis of current technologies, supported by 176 references, and published in a peer-reviewed, high-impact journal.