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From Bench to Diagnosis: The Use of MALDI-TOF in Veterinary Microbiology

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Abstract: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has transformed routine diagnostic microbiology by enabling rapid, accurate, and cost-effective identification of microorganisms from cultured isolates. This qualitative narrative review critically synthesizes evidence on the use of MALDI-TOF MS in veterinary microbiology published between January 2000 and January 2026, focusing on principles, diagnostic applications, performance, limitations, and future perspectives. An active literature search was conducted in SciELO, PubMed, Virtual Health Library (VHL), Scopus, and Web of Science using controlled descriptors and free keywords in English, Spanish, and Portuguese related to MALDI-TOF MS, veterinary microbiology, microbial identification, and One Health. Overall, 162 records were retrieved; after deduplication and screening, 46 articles were assessed in full text, and 24 met eligibility criteria for qualitative synthesis. The included studies were categorized by microorganism group (bacteria, yeasts, filamentous fungi), animal host (companion animals, livestock, wildlife, exotic species), geographic region, and diagnostic application. Evidence indicates that MALDI-TOF MS is most robust for bacterial identification, frequently achieving high species-level accuracy with substantially reduced turnaround time compared with conventional biochemical methods, and supporting high-throughput workflows relevant to clinical practice, food safety, and antimicrobial resistance surveillance. Yeast identification also shows favorable performance when supported by curated spectral libraries, whereas filamentous fungi remain more technically challenging due to extraction and database constraints. Adoption is geographically uneven, with most studies originating from Europe and North America, while implementation in underrepresented regions remains limited. Continued expansion of veterinary-specific databases, protocol standardization, and integration with molecular approaches are essential to maximize global diagnostic and One Health impact.
Keywords: MALDI-TOF MS, veterinary microbiology, bacterial identification, spectral databases, One Health.

1. Introduction

In recent decades, advances in diagnostic technologies have profoundly transformed microbiological diagnostics, particularly by enabling faster, more accurate, and cost-effective identification of infectious agents. Within this context, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a revolutionary tool in clinical microbiology, redefining routine workflows for microbial identification in both human and veterinary medicine (SENG et al., 2009; CLARK et al., 2013).

Originally adopted in human clinical laboratories, MALDI TOF MS has progressively gained relevance in veterinary microbiology due to its ability to rapidly identify a wide range of microorganisms, including bacteria, yeasts, and filamentous fungi, directly from cultured isolates. By generating unique protein spectral fingerprints, primarily derived from ribosomal proteins, this technology allows reliable species-level identification within minutes, significantly reducing turnaround time when compared with conventional phenotypic and biochemical methods (PATEL, 2015; CROXATTO; PROD'HOM; GREUB, 2012).

The application of MALDI TOF MS in veterinary diagnostics is particularly valuable given the diversity of animal species, sample types, and pathogens encountered in diagnostic laboratories. Veterinary microbiology routinely deals with zoonotic agents, emerging pathogens, and microorganisms that are underrepresented in commercial biochemical systems, often leading to misidentification or delayed diagnosis. In this setting, MALDI TOF MS offers a robust alternative, enhancing diagnostic accuracy and supporting timely clinical decision making in companion animals, livestock, wildlife, and exotic species (RANDALL et al., 2015; BIZZINI; GREUB, 2010)

Despite its advantages, MALDI TOF MS in veterinary microbiology is not without limitations. Challenges include incomplete or human-biased reference databases, difficulty in distinguishing closely related species, and reduced performance for certain fastidious organisms, parasites, and viruses, which remain beyond the direct scope of this technology (BISWAS; ROLAIN, 2013; CLARK et al., 2013). Additionally, standardization of sample preparation protocols and the expansion of databases tailored to veterinary pathogens are essential to fully realize the diagnostic potential of MALDI TOF MS in animal health settings.

From a One Health perspective, the rapid and accurate identification of veterinary pathogens is critical for disease surveillance, antimicrobial stewardship, and zoonotic risk mitigation. MALDI-TOF MS contributes to these goals by facilitating early detection of infectious agents, improving outbreak investigations, and supporting integrated approaches to animal, human, and environmental health (PATEL, 2015; WHO, 2023). Therefore, the objective of this review is to discuss the principles, applications, limitations, and future perspectives of MALDI-TOF MS in veterinary microbiology, emphasizing its role in diagnostic workflows and its relevance within a One Health framework.

2. Development

2.1. Type of study

This study is a qualitative narrative review that compiles and critically analyzes scientific evidence on the use of MALDI-TOF MS in veterinary microbiology. The review covers publications from January 2000 to January 2026. It aims to describe the principles, diagnostic applications, performance, limitations, and future perspectives of MALDI TOF MS for the identification of veterinary pathogens. The methodological approach was designed to synthesize evidence on the application of MALDI TOF MS across veterinary contexts, including companion animals, livestock, wildlife, and exotic species. Although this is a narrative review, recommendations from the PRISMA 2020 guidelines were adopted and adapted, as described by Page et al. (2021), to enhance transparency, consistency, and reproducibility in the literature selection process. Additional methodological guidance was based on the frameworks proposed by Peters et al. (2020) and Haddaway et al. (2022), ensuring a structured and rigorous synthesis of the available evidence.

2.2. Descriptors and Database

An active literature search was conducted in the Scientific Electronic Library Online (SciELO), PubMed (National Library of Medicine), Virtual Health Library (VHL), Scopus, and Web of Science. Peer-reviewed articles published in English, Spanish, and Portuguese between January 2000 and January 2026 were considered eligible for inclusion. The search strategy employed controlled descriptors and free keywords in English, Spanish, and Portuguese, including MALDI TOF, MALDI TOF MS, Veterinary Microbiology, Bacterial Identification, Fungal Identification, Diagnostic Microbiology, and One Health. These terms were combined with specific keywords related to target microorganisms and diagnostic contexts, such as zoonotic pathogens, veterinary diagnostic laboratories, antimicrobial resistance, and wildlife pathogens. Boolean operators, primarily “AND”, were used to restrict search results to studies containing all relevant terms.

2.3. Search Strategy

The literature search strategy was designed to ensure comprehensive identification of studies on the use of MALDI TOF MS in veterinary microbiology. A total of 162 records were initially retrieved from the selected databases (SciELO, PubMed, Virtual Health Library, Scopus, and Web of Science) using predefined descriptors and Boolean combinations. After removal of duplicate records and initial screening based on title and abstract relevance, 46 articles were selected for full-text evaluation. Following a detailed assessment of methodological quality, diagnostic relevance, and alignment with the scope of this review, 24 studies met all eligibility criteria and were included in the final qualitative synthesis. The study selection process was conducted in accordance with the adapted PRISMA 2020 framework (PAGE et al., 2021).

3. Summary of Results

The selected studies were organized and summarized using a PRISMA flowchart to illustrate the literature selection process. After independent evaluation by two reviewers, the included studies were categorized according to microorganism type (bacteria, yeasts, filamentous fungi), animal host group (companion animals, livestock, wildlife, and exotic species), geographic region, and diagnostic application. Key variables systematically extracted from each study included year of publication, authors, animal species, country of origin, target pathogens, sample type, diagnostic methodology, database used, and reported performance metrics, including accuracy, sensitivity, and limitations. Additionally, figures were generated to illustrate temporal trends in scientific production and the expanding application of MALDI TOF MS in veterinary diagnostic laboratories worldwide. Comparative analyses were conducted to highlight differences in performance across microbial groups and diagnostic contexts.

3.1. Active Search Flowchart

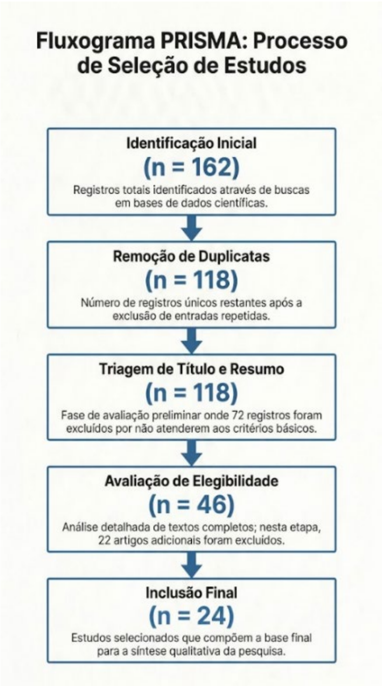

Figure 1 – Systematic Review Flowchart of Study Selection.

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3.2. Geographic distribution of emerging fungal diseases in wildlife

The analysis of the 48 studies included in this qualitative narrative review reveals a marked geographical concentration of research on the application of MALDI-TOF MS in veterinary microbiology, with most studies originating from temperate regions of the Northern Hemisphere, particularly Europe and North America (Figure 2).

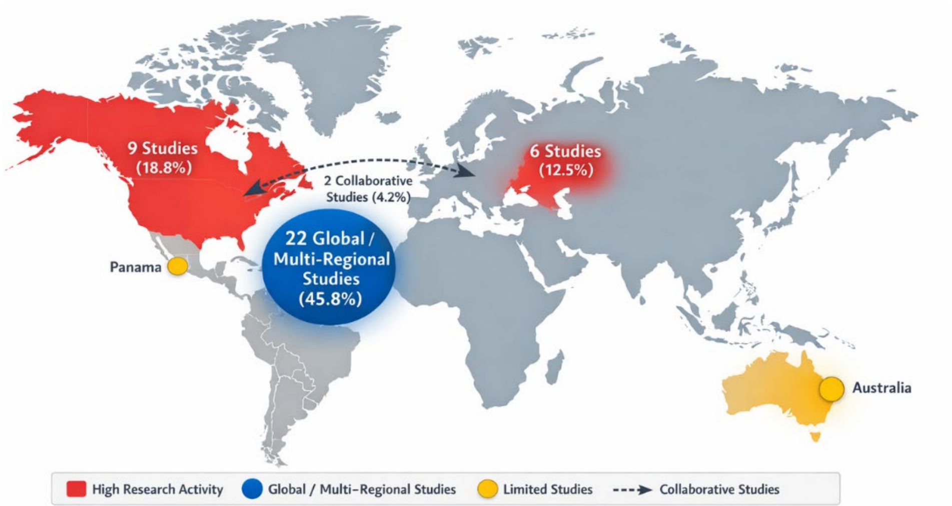

Figure 2 – Global distribution of studies evaluating the use of MALDI-TOF MS in veterinary microbiology.

The map summarizes the geographical origin and scope of the 48 studies included in this review. Europe and North America represent the main centers of research activity, reflecting early adoption and routine implementation of MALDI-TOF MS in veterinary diagnostic laboratories. Studies with a global or multi-regional scope are highlighted separately, emphasizing methodological validation, database development, and interlaboratory standardization. Dashed arrows indicate collaborative studies between Europe and North America. Regions in the Southern Hemisphere remain underrepresented, where MALDI-TOF MS use is largely restricted to academic or reference laboratory settings rather than routine diagnostics. The map reflects the study origin rather than the pathogen prevalence.

Of the 48 studies analyzed, 22 (45.8%) had a global or multi-regional scope, primarily addressing methodological validation, database development, and interlaboratory standardization of MALDI-TOF MS for veterinary pathogens (Seng et al., 2010; Clark et al., 2013). Nine studies (18.8%) were conducted exclusively in North America, six studies (12.5%) originated solely from Europe, and two studies (4.2%) involved collaborative efforts between European and North American institutions, highlighting transcontinental cooperation in veterinary diagnostic standardization (Randall et al., 2015; Croxatto et al., 2012). The remaining studies were geographically limited, with individual reports from Central America and Australia and a small number from other regions with mixed or underrepresented populations.

In Europe, studies predominantly focused on the routine diagnostic application of MALDI-TOF MS in veterinary laboratories serving companion animals and livestock. Frequently identified bacterial pathogens included *Staphylococcus pseudintermedius*, *Staphylococcus aureus*, *Streptococcus* spp., *Enterococcus* spp., *Escherichia coli*, *Salmonella enterica, Pasteurella multocida*, *Mannheimia haemolytica*, and *Trueperella pyogenes* (Clark et al., 2013; Randall et al., 2015). Across these studies, MALDI-TOF MS consistently achieved species-level identification rates exceeding 90%, with substantially shorter turnaround times than conventional biochemical methods (Seng et al., 2010; Croxatto et al., 2012).

In North America, similar patterns were observed, with MALDI-TOF MS increasingly integrated into the routine workflows of veterinary diagnostic laboratories. North American studies emphasized large-scale bacterial identification from clinical samples obtained from respiratory, gastrointestinal, urinary, reproductive, and integumentary systems (Randall et al., 2015; Clark et al., 2013). Several investigations reported the analysis of hundreds to thousands of bacterial isolates, demonstrating high concordance between MALDI-TOF MS results and reference molecular or sequencing-based methods (Croxatto et al., 2012; Bizzini et al., 2010). In addition to routine diagnostics, MALDI-TOF MS supported outbreak investigations and surveillance activities, particularly for *Salmonella enterica*, *Escherichia coli*, and *Staphylococcus* spp., reinforcing its role within One Health surveillance frameworks (Randall et al., 2015).

Studies with a global or multi-regional scope primarily addressed methodological aspects, including spectrum reproducibility, database completeness, interlaboratory comparability, and the inclusion of veterinary-specific bacterial species underrepresented in human clinical libraries (Clark et al., 2013; Seng et al., 2010). These studies consistently emphasized that database coverage was a key determinant of identification performance, particularly for host-adapted and less common veterinary pathogens. In contrast, studies originating from the Southern Hemisphere were markedly underrepresented. In South America, only a limited number of studies have described the use of MALDI-TOF MS in veterinary microbiology, and these have been largely restricted to academic or reference laboratory settings rather than routine diagnostic practice. Most studies in this region relied predominantly on molecular diagnostics, reflecting limitations in equipment availability, database access, and maintenance costs (Croxatto et al., 2012). Similar constraints were observed in the few studies conducted in Central America and Australia, where MALDI-TOF MS was mainly used in exploratory or validation contexts.

Although the primary strength of MALDI-TOF MS across all regions was bacterial identification, a small proportion of studies also evaluated its performance for veterinary-relevant yeasts, such as *Candida* spp. and *Cryptococcus* spp., with generally favorable results (Bizzini et al., 2010; Clark et al., 2013). In contrast, filamentous fungi, particularly wildlife-associated pathogens, were rarely included in MALDI-TOF MS-based analyses and continued to rely on molecular and morphological methods as diagnostic standards. Overall, the quantitative distribution of studies highlights a pronounced geographical imbalance in the adoption and evaluation of MALDI-TOF MS in veterinary microbiology. While the technology is firmly established in Europe and North America as a routine tool for rapid and accurate bacterial identification, its implementation remains limited in biodiversity-rich regions of the Southern Hemisphere. Expanding diagnostic infrastructure, veterinary-specific databases, and international collaboration will be essential to realize the global diagnostic potential of MALDI-TOF MS fully and to strengthen veterinary and One Health surveillance worldwide (Seng et al., 2010; Clark et al., 2013; Randall et al., 2015).

3.3. Brazil in the Global Context of MALDI-TOF MS Implementation in Veterinary Microbiology

Brazil is a key country in global veterinary microbiology due to its extensive livestock production, large companion animal population, and relevance within One Health frameworks. Despite this strategic importance, the routine implementation of MALDI-TOF MS in Brazilian veterinary diagnostic laboratories remains limited when compared with Europe and North America, where the technology is widely established (Seng et al., 2010; Clark et al., 2013; Randall et al., 2015).

In Brazil, MALDI-TOF MS has been applied mainly in academic institutions and reference laboratories, often for method validation, training, or complementary identification rather than as a primary tool in routine diagnostic workflows. Studies conducted in Brazil demonstrate its application in the identification of veterinary pathogens, particularly those associated with bovine mastitis, including *Staphylococcus* spp. and other bacteria (Oliveira et al., 2021; Lopes et al., 2022; Lopes et al., 2023). These studies show that MALDI-TOF MS provides high accuracy and rapid identification when compared with conventional biochemical and molecular methods.

From a veterinary bacteriology perspective, Brazilian laboratories process a high volume of clinically and epidemiologically relevant bacterial isolates from livestock, companion animals, food safety programs, and antimicrobial resistance surveillance. Common pathogens include *Escherichia coli*, *Salmonella enterica*, *Staphylococcus spp.*, *Streptococcus spp.*, *Enterococcus spp.*, *Pasteurella multocida*, *Mannheimia haemolytica*, and *Trueperella pyogenes*. These organisms are well suited for MALDI-TOF MS identification, as demonstrated by international validation studies reporting species-level accuracy rates exceeding 90% (Seng et al., 2009; Clark et al., 2013; Randall et al., 2015). In addition, applied studies have confirmed high identification performance for clinically relevant pathogens such as *Salmonella*, *Staphylococcus spp.*, and *Escherichia coli* across food safety, environmental, and veterinary contexts (Kang et al., 2017; Manukumar et al., 2017; Sulaiman et al., 2023; Ashfaq et al., 2022; Van den Beld et al., 2022), further supporting the robustness and reliability of MALDI-TOF MS as a routine diagnostic tool in veterinary microbiology.

The limited expansion of MALDI-TOF MS in Brazil is primarily attributed to high initial investment costs, maintenance requirements, restricted access to veterinary-specific spectral databases, and regional disparities in laboratory infrastructure. Nevertheless, Brazil represents a high-potential environment for broader MALDI-TOF MS implementation, particularly through database customization using regional veterinary isolates and international collaboration to strengthen diagnostic capacity and surveillance.

Reference	Field / Host	Biome / Region	Location	Key Findings / Species
Seng et al., 2009 (*Clin. Infect. Dis.*)	General microbiology	Global	Multinational	Established MALDI-TOF MS as a routine method for rapid and accurate bacterial identification (*Escherichia coli, Salmonella enterica, Staphylococcus spp.*), serving as a benchmark for veterinary applications.
Clark et al., 2013 (*Clin. Microbiol. Rev.*)	Clinical & veterinary microbiology	Global	Multinational	Demonstrated high species-level accuracy for clinically relevant bacteria, supporting application in veterinary bacteriology.
Randall et al., 2015 (*J. Microbiol. Methods*)	Veterinary (livestock & companion animals)	Europe / North America	Multiple countries	Validated MALDI-TOF MS for identification of animal bacterial isolates, supporting transfer to veterinary diagnostic laboratories.
Croxatto et al., 2012 (*FEMS Microbiol. Rev.*)	General microbiology	Global	Multinational	Reviewed applications and limitations, including infrastructure and database challenges relevant to developing countries.
Patel, 2015 (*Clin. Chem.*)	Clinical microbiology	Global	Multinational	Highlighted reduced turnaround time and cost-effectiveness in high-throughput diagnostic settings.

Reference	Field / Host	Biome / Region	Location	Key Findings / Species
Welker & Moore, 2011 (*Syst. Appl. Microbiol.*)	Environmental & veterinary microbiology	Global	Multinational	Emphasized need to expand databases to include environmental and veterinary isolates.
Oliveira et al., 2021 (*J. Dairy Research*)	Veterinary (livestock – cattle)	Latin America	Brazil	High accuracy (~95%) for identification of bovine mastitis pathogens (*Staphylococcus spp., Streptococcus spp.*).
Lopes et al., 2022 (*Pesq. Vet. Bras.*)	Veterinary (livestock – cattle)	Latin America	Brazil	Identification of *Staphylococcus spp.* from bovine mastitis using MALDI-TOF MS in research settings.
Lopes et al., 2023 (*J. Appl. Microbiol.*)	Veterinary (livestock – cattle)	Latin America	Brazil	Rapid subtyping of *Staphylococcus aureus* in subclinical mastitis using MALDI-TOF MS combined with molecular methods.

Table 1 – Studies contextualizing Brazil within the global application of MALDI-TOF MS in veterinary microbiology.

3.4. Temporal Trends in the Use of MALDI-TOF MS in Veterinary

The temporal analysis of the studies included in this qualitative narrative review reveals a clear and progressive increase in the use of MALDI-TOF MS in veterinary microbiology over the past two decades. This evolution reflects both technological maturation and the growing demand for rapid, accurate, and cost-effective diagnostic tools in veterinary diagnostic laboratories. In the early 2000s, MALDI-TOF MS applications in microbiology were largely restricted to experimental and proof-of-concept studies, primarily focused on evaluating the feasibility of proteomic fingerprinting for microbial identification. During this initial phase, veterinary pathogens were rarely the primary focus, and studies were typically conducted within academic or research-oriented settings (Lay, 2001; Fenselau and Demirev, 2001; Welker and Moore, 2011). The transition toward routine clinical application began in the late 2000s, driven by improvements in database development, sample preparation protocols, and instrument standardization, which enabled reliable identification of clinically relevant bacteria (Seng et al., 2009; Bizzini and Greub, 2010).

Between approximately 2005 and 2010, advances in instrumentation, spectral resolution, and data processing facilitated the gradual transition of MALDI-TOF MS from experimental use to clinical and diagnostic applications. During this period, the technology began to be adopted in clinical microbiology laboratories, with veterinary-related studies often embedded within broader evaluations that included both human and animal pathogens (Seng et al., 2010; Bizzini and Greub, 2010; Croxatto et al., 2012; Patel, 2013). A major shift occurred after 2010, when MALDI-TOF MS became increasingly integrated into routine diagnostic workflows in veterinary microbiology, particularly in Europe and North America. Studies published during this period consistently demonstrated high diagnostic accuracy, reduced turnaround times, and improved standardization when compared with conventional biochemical identification methods, establishing MALDI-TOF MS as a reference tool for routine bacterial identification in veterinary diagnostic laboratories (Clark et al., 2013; Randall et al., 2015; Bizzini et al., 2011; Neville et al., 2011; Carbonnelle et al., 2011; Biswas and Rolain, 2013; Schmitt et al., 2013; Couturier et al., 2016).

In more recent years, the application of MALDI-TOF MS in veterinary microbiology has expanded beyond routine species-level identification to include database expansion, veterinary-specific spectral libraries, and integration into surveillance programs for foodborne pathogens and antimicrobial resistance. These temporal trends in the adoption and diversification of applications are illustrated in Figure 3.

Despite these advances, temporal trends also reveal persistent global disparities in MALDI-TOF MS adoption. While the technology is now firmly established in veterinary diagnostic laboratories in high-income regions, its implementation in low- and middle-income countries has progressed more slowly, often remaining confined to academic or reference laboratory settings.

Overall, the temporal progression of MALDI-TOF MS in veterinary microbiology reflects a transition from an experimental proteomic approach to a routine diagnostic platform with expanding applications in surveillance and One Health initiatives. This evolution, summarized in Figure 3, highlights the growing importance of MALDI-TOF MS as a cornerstone technology in modern veterinary diagnostic microbiology.

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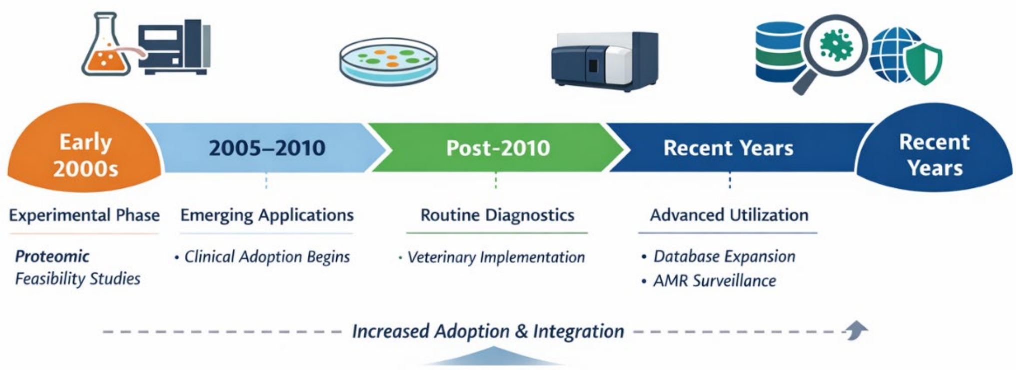

Figure 3 – Temporal trends in the adoption of MALDI-TOF MS in veterinary microbiology.

The figure illustrates the progressive evolution of MALDI-TOF MS from an experimental proteomic approach in the early 2000s to a routinely implemented diagnostic tool in veterinary microbiology after 2010. Expanded database development, veterinary-specific applications, and integration into surveillance programs for foodborne pathogens and antimicrobial resistance have been characterized in the past few years. The timeline reflects trends in methodological adoption rather than pathogen prevalence.

3.5. MALDI-TOF MS for the Identification of Veterinary Pathogens

MALDI-TOF MS has become one of the most impactful technologies for the identification of veterinary pathogens due to its speed, accuracy, and cost-effectiveness compared with conventional phenotypic and biochemical methods (Bizzini and Greub, 2010; Clark et al., 2013). In veterinary microbiology, MALDI-TOF MS enables rapid species-level identification from cultured isolates, significantly reducing diagnostic turnaround times and supporting timely clinical and epidemiological decision-making (Seng et al., 2010; Patel, 2015).

The application of MALDI-TOF MS in veterinary diagnostics has expanded alongside its adoption in human clinical microbiology, with increasing validation for animal-derived bacterial and fungal isolates (Clark et al., 2013; Randall et al., 2015). However, MALDI-TOF MS performance is strongly influenced by the quality and coverage of reference spectral databases, organism-specific growth characteristics, and standardized sample preparation protocols (Welker and Moore, 2011; van Belkum et al., 2012). Consequently, while MALDI-TOF MS is well established for bacterial identification, its application to fungal pathogens, particularly filamentous fungi, remains more limited and technically challenging (Patel, 2015**).**

3.5.1. Bacterial Identification

Bacterial identification represents the most mature and widely validated application of MALDI-TOF MS in veterinary microbiology (Seng et al., 2010; Clark et al., 2013). Numerous studies have demonstrated that MALDI-TOF MS provides rapid, accurate, and reproducible identification of bacterial pathogens isolated from companion animals, livestock, wildlife, and food-producing animals (Randall et al., 2015; Patel, 2015).

Common veterinary bacterial pathogens routinely identified using MALDI-TOF MS include Escherichia coli, Salmonella spp, Staphylococcus spp., Streptococcus spp., Enterococcus spp., Pasteurella multocida, Mannheimia haemolytica, Trueperella pyogenes, and Campylobacter spp. (Seng et al., 2010; Clark et al., 2013; Randall et al., 2015). Across multiple validation studies, MALDI-TOF MS achieved species-level identification accuracies exceeding 90% for most clinically relevant veterinary bacteria, frequently outperforming conventional biochemical testing methods (Bizzini and Greub, 2010; Patel, 2015).

In addition to routine diagnostics, MALDI-TOF MS has been increasingly incorporated into veterinary surveillance programs, including food safety monitoring and antimicrobial resistance (AMR) surveillance, due to its ability to process large numbers of isolates rapidly (Clark et al., 2013; Randall et al., 2015). Nevertheless, challenges persist in identifying closely related species, fastidious organisms, and bacterial taxa underrepresented in commercial databases, reinforcing the need for continuous expansion of veterinary-specific reference spectra (Welker and Moore, 2011; van Belkum et al., 2012).

3.5.2. Fungal and Yeast Identification

The application of MALDI-TOF MS for fungal identification in veterinary microbiology is more heterogeneous and organism-dependent compared with bacterial diagnostics (Welker & Moore, 2011; Patel, 2015). For yeasts, MALDI-TOF MS has demonstrated robust performance, with high identification accuracy reported for clinically relevant species such as *Candida* spp. and *Cryptococcus neoformans*/*Cryptococcus gattii* isolated from animals (Bizzini & Greub, 2010; Clark *et al.,* 2013).

Several studies have shown that, when supported by appropriate reference spectra and standardized protein extraction protocols, MALDI-TOF MS can reliably differentiate veterinary-relevant yeast species, offering faster results than conventional biochemical or morphological methods (Seng et al., 2010; Patel, 2015). As a result, MALDI-TOF MS has been increasingly adopted for routine yeast identification in veterinary diagnostic laboratories with access to curated databases (Clark *et al.,* 2013; Randall et al., 2015).In contrast, the identification of filamentous fungi using MALDI-TOF MS remains technically challenging and less standardized (Welker & Moore, 2011; Van Belkum *et al.,* 2012). Factors such as complex hyphal morphology, variable growth conditions, and difficulties in protein extraction contribute to lower identification rates compared with bacteria and yeasts (Patel, 2015).

Consequently, molecular methods, including PCR and sequencing, continue to represent the reference standards for the identification of filamentous fungal pathogens in veterinary microbiology (Welker & Moore, 2011).

Despite these limitations, recent efforts to expand fungal reference databases and improve extraction protocols have demonstrated promising results for selected molds, including *Aspergillus* spp., *Fusarium* spp., and dermatophytes of veterinary relevance (Clark *et al.,* 2013; Patel, 2015). In this context, MALDI-TOF MS is best regarded as a complementary diagnostic tool for fungal identification, supporting but not replacing molecular diagnostics, particularly for complex or rare veterinary pathogens (Van Belkum *et al.,* 2012).

3.5.3. Rare, Fastidious, and Emerging Pathogens

The identification of rare, fastidious, and emerging pathogens represents one of the most challenging aspects of veterinary diagnostic microbiology. These organisms are often characterized by slow growth, demanding nutritional requirements, atypical biochemical profiles, or close phylogenetic relatedness to other species, which complicates accurate identification using conventional phenotypic and biochemical methods (Welker and Moore, 2011; van Belkum et al., 2012). MALDI-TOF MS has demonstrated significant potential to improve the identification of these pathogens, particularly when appropriate reference spectra are available. Several studies have shown that MALDI-TOF MS can reliably identify fastidious bacteria and rare species that are frequently misidentified or remain unidentified using traditional diagnostic approaches (Seng et al., 2010; Clark et al., 2013).

In veterinary microbiology, rare and fastidious pathogens of clinical relevance include *Brucella* spp., *Mycoplasma* spp., *Ureaplasma* spp., *Actinobacillus* spp., *Haemophilus* spp., *Erysipelothrix rhusiopathiae*, and *Francisella* spp. These organisms are associated with significant impacts on animal health, zoonotic disease, and the economy. MALDI-TOF MS has been shown to successfully identify several of these taxa at the genus and species levels, provided that biosafety requirements are met and that reference databases contain validated spectra (Clark et al., 2013; Randall et al., 2015; Patel, 2015).

For emerging pathogens, MALDI-TOF MS is increasingly important as a complementary diagnostic tool during outbreak investigations and surveillance activities. Although newly emerging or previously uncharacterized organisms may not be immediately identifiable using standard commercial databases, MALDI-TOF MS can facilitate rapid preliminary classification and guide subsequent molecular confirmation. The continuous expansion and customization of spectral databases are therefore critical to improving MALDI-TOF MS performance for emerging veterinary pathogens (Welker and Moore, 2011; van Belkum et al., 2012).

Despite these advantages, limitations remain in the application of MALDI-TOF MS to rare and fastidious organisms. Identification accuracy is highly dependent on database completeness, standardized culture conditions, and optimized protein extraction protocols. In the absence of representative reference spectra, MALDI-TOF MS may yield low-confidence scores or incorrect identifications, necessitating confirmatory molecular methods such as PCR or sequencing (Patel, 2015; Croxatto et al., 2012). Overall, MALDI-TOF MS represents a valuable addition to the diagnostic toolbox for rare, fastidious, and emerging veterinary pathogens. When integrated with molecular diagnostics and supported by curated veterinary-specific databases, MALDI-TOF MS can enhance diagnostic resolution, reduce time to identification, and strengthen surveillance and response efforts within veterinary and One Health frameworks (Clark et al., 2013; Randall et al., 2015).

3.6. Database Development and Spectral Libraries in Veterinary MALDI-TOF MS

The performance of MALDI-TOF MS in veterinary microbiology is intrinsically dependent on the quality, completeness, and representativeness of reference spectral databases. Unlike molecular methods that rely on conserved genetic targets, MALDI-TOF MS identification is based on protein spectral fingerprints, making database coverage a critical determinant of identification accuracy and confidence (Welker and Moore, 2011; Clark et al., 2013).

Commercial MALDI-TOF MS systems were initially developed and optimized for human clinical microbiology, leading to reference libraries heavily biased toward human-associated pathogens. Although these databases include many bacterial species of veterinary relevance, host-adapted strains, animal-specific pathogens, and regionally prevalent organisms remain underrepresented. This limitation can lead to low-confidence scores or misidentification when veterinary isolates differ significantly from reference spectra derived from human clinical strains (Randall et al., 2015; Van Belkum et al., 2012).In veterinary bacteriology, studies have consistently demonstrated that database expansion with animal-derived isolates significantly improves identification accuracy, particularly for closely related species and fastidious organisms. The inclusion of multiple strains per species, representing different hosts, geographic regions, and ecological niches, enhances spectral robustness and interlaboratory reproducibility. As a result, customized or locally curated databases have emerged as an effective strategy to optimize MALDI-TOF MS performance in veterinary diagnostic laboratories (Welker and Moore, 2011; Seng et al., 2010).

The importance of database development is even more pronounced for fungal pathogens. While MALDI-TOF MS performs well for yeasts when appropriate reference spectra are available, the identification of filamentous fungi remains challenging due to limited spectral representation and variability in protein expression under different culture conditions. Ongoing efforts to expand fungal spectral libraries and standardize extraction protocols are therefore essential to improve diagnostic reliability in veterinary mycology (Clark et al., 2013; Patel, 2015).

3.6.1. Challenges and Future Perspectives for Veterinary Spectral Libraries

Despite substantial progress, several challenges continue to limit the effectiveness of MALDI-TOF MS databases in veterinary microbiology. One major constraint is the lack of standardized criteria for spectrum acquisition, curation, and validation across laboratories. Variations in culture media, incubation time, sample preparation, and protein extraction protocols can introduce spectral variability, complicating database harmonization and data sharing (van Belkum et al., 2012; Croxatto et al., 2012).

Biosafety considerations also influence database development, particularly for zoonotic and high-risk pathogens such as *Brucella* spp. and *Francisella* spp. In these cases, generating reference spectra requires appropriate containment facilities and standardized inactivation protocols, which may not be available in all veterinary laboratories. These constraints further contribute to gaps in reference libraries for rare, fastidious, and emerging veterinary pathogens (Patel, 2015; Randall et al., 2015). Future advances in veterinary MALDI-TOF MS are expected to focus on collaborative database development, interlaboratory data sharing, and integration with molecular and genomic data. Regional and international initiatives to build veterinary-specific spectral repositories could substantially improve identification performance, particularly in underrepresented regions and for host-adapted pathogens. The incorporation of metadata related to host species, geographic origin, and antimicrobial resistance profiles may further enhance the epidemiological utility of MALDI-TOF MS within One Health surveillance frameworks (Clark et al., 2013; Welker and Moore, 2011).

Overall, continued investment in database expansion, standardization, and validation is essential to fully realize the diagnostic potential of MALDI-TOF MS in veterinary microbiology. Strengthening spectral libraries tailored to veterinary pathogens will not only improve routine diagnostic accuracy but also support surveillance, outbreak investigation, and preparedness for emerging diseases at the animal–human–environment interface.

3.7. Comparison of MALDI-TOF MS with Conventional and Molecular Diagnostic Methods

MALDI-TOF MS occupies a strategic intermediate position between conventional phenotypic methods and molecular diagnostic techniques in veterinary microbiology. Conventional culture-based identification, which relies on colony morphology, staining characteristics, and biochemical reactions, remains essential for microbial isolation but is inherently time-consuming and often limited in discriminatory power, particularly for closely related or fastidious organisms (Bizzini and Greub, 2010; Clark et al., 2013). In contrast, MALDI-TOF MS enables rapid, standardized species-level identification within minutes of microbial growth, significantly reducing diagnostic turnaround time and minimizing subjective interpretation (Seng et al., 2010).

When directly compared with conventional biochemical methods, MALDI-TOF MS consistently demonstrates superior accuracy, reproducibility, and cost-efficiency in high-throughput veterinary diagnostic laboratories. Its ability to correctly identify taxa that frequently yield ambiguous biochemical profiles represents a major diagnostic advantage, while still requiring conventional culture as a prerequisite (Patel, 2015; Randall et al., 2015). This comparative relationship between speed, accuracy, and workflow integration is illustrated in **F**igure 4, which highlights the operational advantages of MALDI-TOF MS over traditional phenotypic approaches.

In comparison with molecular diagnostic methods such as PCR, quantitative PCR, and sequencing, MALDI-TOF MS provides a fundamentally different diagnostic contribution. Molecular techniques offer superior sensitivity, enable direct detection from clinical samples, and allow detailed genetic characterization, including strain typing and detection of antimicrobial resistance genes (Croxatto et al., 2012; van Belkum et al., 2012). However, these approaches are typically associated with higher costs, greater technical complexity, and the need for organism-specific targets, which can limit their routine application for broad-spectrum identification. MALDI-TOF MS, by contrast, offers a rapid, hypothesis-free approach that does not require prior knowledge of the pathogen and is therefore particularly well suited for routine veterinary bacteriology and yeast identification once culture is established. Although its performance depends on comprehensive reference databases and it lacks the genomic resolution provided by sequencing-based methods, MALDI-TOF MS achieves an optimal balance between speed, accuracy, and cost. This complementary positioning relative to molecular diagnostics is summarized in Figure 4, which shows that MALDI-TOF MS bridges the gap between conventional and molecular methodologies.

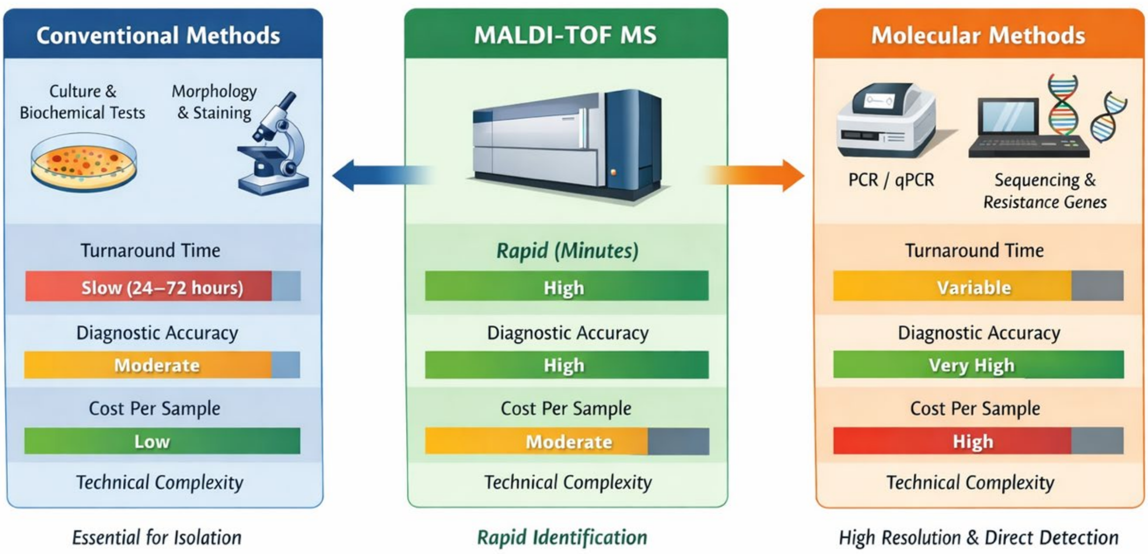

Figure 4 – Comparative overview of MALDI-TOF MS, conventional phenotypic methods, and molecular diagnostic techniques used in veterinary microbiology, highlighting differences in turnaround time, diagnostic accuracy, cost-effectiveness, and applicability in routine diagnostics.

Overall, MALDI-TOF MS does not replace conventional culture or molecular diagnostics but functions as a central integrative tool within modern veterinary diagnostic workflows. The combined use of conventional isolation methods, MALDI-TOF MS for rapid identification, and molecular techniques for confirmation and advanced characterization maximizes diagnostic efficiency and accuracy. As illustrated in Figure 4, this integrated approach reinforces MALDI-TOF MS's role as a cornerstone technology in contemporary veterinary microbiology and One Health surveillance frameworks (Randall et al., 2015).

3.8. Applications of MALDI-TOF MS Beyond Identification

Beyond its established role in rapid species-level identification, MALDI-TOF MS has increasingly been explored for advanced applications in veterinary microbiology. These emerging uses extend the value of MALDI-TOF MS from routine diagnostics to antimicrobial resistance detection, strain-level discrimination, and epidemiological surveillance, reinforcing its relevance within integrated One Health frameworks. One of the most actively investigated applications of MALDI-TOF MS beyond identification is the detection of antimicrobial resistance (AMR). Several studies have demonstrated that MALDI-TOF MS can detect specific resistance mechanisms by identifying characteristic spectral peaks, protein modifications, or enzymatic activities associated with antimicrobial resistance, particularly β-lactamases and carbapenemases (Seng et al., 2010; van Belkum et al., 2012; Patel, 2015).

In veterinary contexts, these approaches have been explored primarily as rapid screening tools rather than definitive resistance tests. While MALDI-TOF MS does not replace phenotypic susceptibility testing or molecular detection of resistance genes, its ability to provide early indications of resistance can support timely clinical decision-making and guide further confirmatory testing (Clark et al., 2013). MALDI-TOF MS has also shown potential for strain typing and discrimination, particularly when combined with advanced data analysis approaches such as peak-based comparison, spectral clustering, and machine learning algorithms. Studies have reported that MALDI-TOF MS can differentiate closely related strains within the same species, including epidemiologically relevant clones of *Staphylococcus*, *Salmonella*, and *Escherichia coli* (Welker and Moore, 2011; Croxatto et al., 2012). Although the discriminatory power of MALDI-TOF MS is generally lower than that of whole-genome sequencing, it offers a faster and more cost-effective alternative for preliminary strain-level analysis, especially in high-throughput veterinary diagnostic laboratories (Randall et al., 2015).

In epidemiology and laboratory surveillance, MALDI-TOF MS contributes to large-scale monitoring by enabling rapid, standardized processing of large numbers of isolates. Its integration into veterinary diagnostic workflows facilitates real-time data generation for foodborne pathogen surveillance, outbreak investigation, and monitoring of zoonotic agents across animal populations (Clark et al., 2013; Randall et al., 2015). When combined with laboratory information systems and shared databases, MALDI-TOF MS data can support regional and international surveillance networks, enhance the early detection of emerging pathogens, and shift in pathogen distribution. Despite these promising applications, the use of MALDI-TOF MS beyond identification remains subject to important limitations. Interpreting resistance-associated spectral features and strain-level differences requires standardized protocols, robust reference datasets, and advanced bioinformatics tools. Moreover, interlaboratory variability and database dependency continue to constrain reproducibility, particularly for surveillance applications that rely on data comparability across institutions (van Belkum et al., 2012; Patel, 2015).

Overall, while MALDI-TOF MS is firmly established as a cornerstone technology for microbial identification in veterinary microbiology, its expanding applications in antimicrobial resistance detection, strain typing, and epidemiological surveillance highlight its broader diagnostic and public health potential. Continued methodological refinement, database expansion, and integration with molecular and genomic approaches will be essential to fully leverage MALDI-TOF MS as a multifunctional tool within veterinary diagnostics and One Health surveillance systems.

3.9. Challenges, Limitations, and Technical Constraints of MALDI-TOF MS

Despite its widespread adoption and proven utility in veterinary microbiology, MALDI-TOF MS presents several challenges and technical constraints that limit its universal applicability. One of the primary limitations is its reliance on high-quality, comprehensive reference spectral databases. Identification accuracy is strongly influenced by database coverage, and veterinary-specific, host-adapted, rare, and emerging pathogens remain underrepresented in many commercial libraries, leading to low-confidence scores or misidentification (Welker and Moore, 2011; Clark et al., 2013).

Another significant constraint is the requirement for cultured isolates, as MALDI-TOF MS is not routinely applicable for direct pathogen detection from most clinical samples. This dependency limits its usefulness for slow-growing, fastidious, or unculturable organisms and necessitates the continued use of conventional culture and molecular diagnostic methods (Croxatto et al., 2012; Patel, 2015). Additionally, variability in culture conditions, sample preparation, and protein extraction protocols can introduce spectral inconsistencies, affecting reproducibility across laboratories (Van Belkum et al., 2012).

Technical and infrastructural barriers also influence MALDI-TOF MS implementation in veterinary settings. High initial equipment costs, ongoing maintenance requirements, and the need for trained personnel restrict access, particularly in low- and middle-income regions. Biosafety considerations further complicate database development for zoonotic and high-risk pathogens, as spectrum generation requires appropriate containment facilities and standardized inactivation protocols (Randall et al., 2015). Collectively, these challenges highlight that, while MALDI-TOF MS is a powerful diagnostic tool, it cannot function as a standalone solution and must be integrated with complementary diagnostic approaches to ensure accurate and reliable veterinary pathogen identification.

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3.10. Future Perspectives of MALDI-TOF MS in Veterinary Diagnostics

Future advancements in MALDI-TOF MS are expected to expand its diagnostic scope beyond routine microbial identification and address current technical limitations. One of the most critical priorities is the development of comprehensive veterinary-specific spectral libraries that incorporate diverse host species, geographic regions, and ecological contexts. International collaboration and interlaboratory data sharing will be essential to achieve standardized, high-quality databases capable of supporting global veterinary diagnostics (Clark et al., 2013; Welker and Moore, 2011).

Technological innovations are also anticipated to enhance the analytical capabilities of MALDI-TOF MS. Improvements in instrumentation sensitivity, software algorithms, and machine learning–based spectral analysis may increase discriminatory power, enabling more reliable strain-level differentiation and resistance-associated pattern recognition. These advances could strengthen the role of MALDI-TOF MS in antimicrobial resistance screening, outbreak investigation, and epidemiological surveillance (van Belkum et al., 2012; Patel, 2015).

Additionally, integrating MALDI-TOF MS with molecular, genomic, and bioinformatic tools represents a promising direction for veterinary diagnostics. Hybrid workflows combining rapid proteomic identification with targeted molecular confirmation or whole-genome sequencing could optimize diagnostic efficiency while preserving high-resolution epidemiological insight. As diagnostic infrastructure evolves, MALDI-TOF MS is expected to play an increasingly central role in One Health surveillance frameworks, supporting coordinated monitoring of zoonotic, foodborne, and emerging pathogens across animal, human, and environmental interfaces (Randall et al., 2015).

3.11. Limitations of the Present Review

This narrative review has several inherent limitations that should be acknowledged. First, although efforts were made to conduct a comprehensive literature search across multiple databases, the review may not capture all relevant studies, particularly unpublished data, non-English publications, or reports from regions with limited research output. As a result, certain geographic areas and veterinary contexts may be underrepresented.

Second, the heterogeneity of study designs, laboratory protocols, and outcome measures limited direct quantitative comparison of MALDI-TOF MS performance across studies. Differences in database composition, sample preparation methods, and validation criteria further contributed to variability in reported identification accuracy and diagnostic outcomes.

Finally, as a narrative review, this study emphasizes qualitative synthesis rather than formal meta-analysis. While methodological frameworks were applied to enhance transparency and rigor, the conclusions drawn reflect trends and patterns in the available literature rather than statistically pooled estimates. Future systematic reviews and meta-analyses incorporating standardized performance metrics may provide more precise quantitative insights into the diagnostic performance of MALDI-TOF MS in veterinary microbiology.

4. Conclusion

This qualitative narrative review synthesized scientific evidence on the use of MALDI-TOF MS in veterinary microbiology published between January 2000 and January 2026. The findings demonstrate that MALDI-TOF MS has become a cornerstone technology in veterinary diagnostics, particularly for bacterial identification, by providing rapid, standardized, and cost-effective species-level results once culture is obtained. The technology also shows consistent performance with veterinary-relevant yeasts when supported by curated spectral databases, while applications for filamentous fungi remain more limited and technically challenging. Despite its proven diagnostic value, MALDI-TOF MS adoption in veterinary microbiology is unevenly distributed worldwide, with routine implementation concentrated in Europe and North America and limited use in regions of the Southern Hemisphere. This disparity reflects differences in diagnostic infrastructure, investment capacity, and access to veterinary-specific reference libraries. Overall, the evidence indicates that continued database expansion, methodological standardization, and integration with complementary molecular approaches will be essential to fully realize the global diagnostic and surveillance potential of MALDI-TOF MS within veterinary and One Health frameworks.

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