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Article

The Future of Mycobacterium Tuberculosis Diagnostics: Integrating Mass Spectrometry, AI, and Genomics

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ABSTRACT

Background: Tuberculosis (TB), caused by Mycobacterium tuberculosis, remains a major global health threat, with significant morbidity and mortality exacerbated by the emergence of multidrug-resistant strains and diagnostic challenges in high-burden settings. **Objective**: This review aimed to critically evaluate recent advancements in TB diagnostics, focusing on the integration of mass spectrometry, artificial intelligence, and genomics in comparison to conventional and molecular methods, and to assess their clinical applicability and limitations. **Methods**: A narrative review was conducted by systematically searching PubMed, Scopus, Web of Science, and Google Scholar for English-language peer-reviewed articles published from 2010 to 2023, using structured inclusion and exclusion criteria. Diagnostic performance, comparative accuracy, operational feasibility, and implementation barriers were extracted and synthesized across diverse populations. **Results**: Molecular diagnostics such as Xpert MTB/RIF have substantially improved rapid detection and rifampicin resistance screening, with pooled sensitivities above 90%, while advanced modalities including next-generation sequencing and mass spectrometry offer comprehensive resistance profiling but remain limited by cost and infrastructure requirements. Immunological assays provide high specificity for latent infection but cannot reliably distinguish active disease. Evidence synthesis reveals persistent gaps in pediatric, extrapulmonary, and HIV-positive populations, and underscores the need for large-scale validation and equitable deployment. **Conclusion**: Advanced TB diagnostics offer significant promise but must be strategically integrated with established approaches and rigorously validated in real-world settings to enhance global TB control and patient outcomes.
Keywords: Tuberculosis, molecular diagnostics, mass spectrometry, artificial intelligence, drug resistance, Xpert MTB/RIF, immunological assays, next-generation sequencing, clinical utility.

INTRODUCTION

Tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB), remains a major global health challenge, causing significant morbidity and mortality worldwide. TB was the leading infectious cause of death globally prior to the COVID-19 pandemic, surpassing even HIV/AIDS (1). In 2021 alone, an estimated 10.6 million individuals developed TB, with approximately 1.5 million fatalities, highlighting the continued public health burden (2). The emergence and spread of multidrug-resistant (MDR-TB), extensively drug-resistant (XDR-TB), and totally drug-resistant (TDR-TB) strains have further complicated TB management, particularly in regions with limited diagnostic infrastructure (3,4). Conventional diagnostic methods, including microscopy and culture-based techniques, are widely used due to their cost-effectiveness and established procedures. However, these methods frequently lack the necessary sensitivity and specificity to detect extrapulmonary forms of TB or distinguish active TB from latent infections, thereby delaying appropriate interventions (5,6). Serological assays, historically employed in some clinical settings, have consistently demonstrated poor diagnostic accuracy and are discouraged by the World Health Organization (WHO) due to high rates of false-positive and negative results (7). Furthermore, the accuracy of immunological methods such as tuberculin skin tests (TST) and interferon gamma-release assays (IGRAs) is compromised by prior BCG vaccination and host immunosuppression, contributing to diagnostic ambiguity (8,9).

The global COVID-19 pandemic exacerbated these challenges, significantly disrupting TB control programs, reducing diagnostic availability, and increasing undiagnosed TB cases, particularly in resource-constrained settings (10). Pakistan, which ranks fifth

globally in TB burden, illustrates the urgent need for improved diagnostics given its high prevalence, incidence, and mortality rates of TB—approximately 348, 276, and 34 per 100,000 population respectively—necessitating enhanced public awareness and diagnostic accuracy (11). Recent advancements in molecular diagnostic technologies, such as Xpert MTB/RIF and line probe assays (LPAs), have improved the detection of rifampicin and isoniazid resistance, yet limitations remain concerning broader resistance profiles and detection sensitivity in low bacillary load scenarios (12,13). Emerging diagnostic tools, including mass spectrometry (particularly matrix-assisted laser desorption ionization-time-of-flight mass spectrometry, MALDI-TOF MS), next-generation sequencing (NGS), and artificial intelligence (AI), offer significant promise in addressing these gaps by enabling rapid identification, comprehensive drug susceptibility profiling, and improved predictive capabilities (14–16). Despite these technological advancements, significant barriers persist regarding their integration into routine clinical practice, particularly in low- and middle-income countries (LMICs), which bear the highest TB burden (17).

This narrative review aims to address these gaps by evaluating the recent advancements in TB diagnostics, specifically focusing on the integration of mass spectrometry, AI, and genomic sequencing. It critically assesses their diagnostic accuracy, clinical applicability, and implementation barriers compared to conventional techniques. The objective is to answer the following research question: Do advanced diagnostic methods such as mass spectrometry, artificial intelligence, and next-generation sequencing significantly improve sensitivity, specificity, and clinical utility for diagnosing TB compared to traditional methods, and what implications do these technologies have for adoption in high-burden, resource-limited settings?

MATERIALS AND METHODS

This narrative review was conducted using a structured approach consistent with the SANRA (Scale for the Assessment of Narrative Review Articles) framework, facilitating transparency and reproducibility. A comprehensive literature search was conducted across major scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar. Searches were carried out for publications from January 2010 through December 2023, utilizing carefully selected Medical Subject Headings (MeSH) and keywords such as “Tuberculosis diagnosis,” “Mycobacterium tuberculosis detection,” “molecular diagnostics for tuberculosis,” “mass spectrometry and tuberculosis detection,” “next-generation sequencing in TB,” and “artificial intelligence in TB diagnosis.” These terms were combined using Boolean operators "AND" and "OR" to enhance search specificity and comprehensiveness.

Inclusion criteria encompassed peer-reviewed articles published in English that specifically investigated diagnostic methodologies for TB, including microbiological, molecular, immunological, proteomic, and computational approaches. Articles were selected if they provided empirical data on diagnostic performance metrics, such as sensitivity, specificity, or predictive value, and were directly relevant to clinical or laboratory settings. Exclusion criteria clearly defined and omitted non-peer-reviewed articles, editorials, opinion pieces, studies lacking explicit methodological descriptions, and research not directly related to diagnostic technologies for TB. Animal studies and reviews without original data were also excluded. Article selection involved a two-stage screening process. Initially, titles and abstracts of retrieved articles were independently reviewed by two authors to identify potentially eligible studies. Subsequently, full-text articles were examined independently by the same authors to confirm eligibility based on predefined criteria. Disagreements regarding article inclusion were resolved by discussion to achieve consensus. Data extraction from eligible articles was performed systematically using a predefined standardized extraction form. Key variables extracted included study design, diagnostic methodologies used, study populations, measures of diagnostic accuracy (sensitivity and specificity), limitations reported by the authors, and clinical implications of findings. Data were synthesized and organized into thematic categories to facilitate comparison and evaluation: challenges associated with current diagnostic approaches, advancements in molecular and immunological diagnostic methods, novel emerging technologies (mass spectrometry, NGS, AI-based tools), and recommendations for future diagnostic strategies in TB control. Quality assessment and risk-of-bias evaluation were conducted informally by critically appraising study methodology, diagnostic validation procedures, and consistency of reported outcomes across included studies. This appraisal focused on transparency of reported methods, robustness of statistical analyses, and potential biases in study designs and reporting. Given the nature of this narrative review and its reliance solely on previously published studies, formal ethical approval was not required. Nonetheless, ethical standards for the responsible conduct of literature reviews were strictly followed, ensuring appropriate attribution of all source materials through accurate citations and reference listings.

RESULTS

A wide spectrum of diagnostic approaches for Mycobacterium tuberculosis (MTB) has been evaluated in the literature, ranging from traditional microscopy and culture-based methods to advanced molecular, immunological, and emerging technologies. Their performance characteristics, clinical contexts, and implementation challenges are summarized below, with emphasis on their comparative diagnostic yield and operational feasibility in diverse patient populations. Microscopy, primarily based on the identification of acid-fast bacilli (AFB) using Ziehl-Neelsen (ZN) staining, remains one of the most widely used methods in low-resource settings due to its low cost and rapid turnaround. However, its sensitivity varies considerably by patient population and specimen type, with reported ranges from 25.3% to 81.6% and specificity from 83.4% to 99% when referenced against culture as the standard (1,2). Sensitivity is notably reduced in high-risk groups such as children and HIV-positive individuals due to lower bacterial loads (3). Advances in microscopy, including the adoption of light-emitting diode (LED) fluorescence, have modestly improved diagnostic yield but remain fundamentally limited by their dependence on operator skill and bacillary concentration (4). Microscopy

retains value for assessing infectiousness, monitoring treatment response, and predicting relapse risk, but its inability to detect extrapulmonary TB or distinguish between active and latent infection constrains its utility in comprehensive TB control.

Culture-based methods, including both solid (Lowenstein-Jensen, Middlebrook 7H10/7H11) and liquid media (MGIT, BACTEC), continue to be considered the gold standard for MTB diagnosis due to their superior sensitivity and specificity—often exceeding 95% in clinical studies (5). Culture also enables phenotypic drug susceptibility testing (DST), which is essential for guiding therapy in drug-resistant TB. However, the slow growth rate of MTB extends turnaround times (4–8 weeks for solid, 7–14 days for liquid cultures), and technical requirements such as sample decontamination and biosafety facilities limit scalability in resource-constrained settings (6). Rapid identification of MTB in positive cultures can be achieved using immunochromatographic (ICT) assays that detect the MPT-64 antigen, with pooled specificity and sensitivity estimates of 99.2–100% and 95.8–98.6%, respectively (7). Liquid culture-based DST (e.g., BACTEC MGIT 960) offers results within two weeks and remains the reference for resistance testing, though the need for critical concentration adjustment and minimum inhibitory concentration (MIC) methods remains a topic of international consensus-building (8,9).

Table 1. Comparison of Microscopic and Culture-Based Diagnostic Methods for Tuberculosis

Feature	Microscopy	Culture
Principle	Examines stained slides for acid-fast bacilli (AFB) under a microscope	Grows Mycobacterium tuberculosis from patient samples
Types Used	Ziehl-Neelsen (ZN), Auramine-Rhodamine, LED fluorescence	Solid media (Lowenstein-Jensen, Middlebrook 7H10/7H11); Liquid media (MGIT, BACTEC)
Sensitivity	Variable (25.3–81.6%) Lower in children, HIV+ and extrapulmonary TB	High (>95%) Detects even a few bacilli
Specificity	Moderate to high (83.4–99%) May require confirmation	High (>95%) Considered gold standard
Turnaround Time	Minutes to hours	Liquid: 7–14 days Solid: 4–8 weeks
Cost	Low to moderate	High (infrastructure, labor, supplies)
Advantages	Rapid, low cost, minimal equipment. Useful for initial screening	Most sensitive and specific; Enables drug susceptibility testing
Limitations	Operator dependent. Low sensitivity in paucibacillary disease; Cannot distinguish live/dead bacilli; Poor for EPTB or children	Slow; Requires biosafety and lab infrastructure; Potential for contamination

Molecular diagnostics have transformed TB detection and drug resistance profiling by reducing time to diagnosis and enhancing sensitivity, particularly in smear-negative or paucibacillary disease. The Xpert MTB/RIF assay, a real-time PCR platform targeting the rpoB gene for MTB and rifampicin resistance, demonstrates pooled sensitivities of 98–99.8% in smear-positive, culture-confirmed cases and approximately 90% in smear-negative, culture-positive cases, with specificity consistently above 98% (10,11). Xpert MTB/RIF Ultra, its successor, incorporates multicopy targets (IS6110 and IS1081) to improve sensitivity, though a minor reduction in specificity has been reported (12). Importantly, these assays do not detect isoniazid resistance, potentially missing a subset of multidrug-resistant TB (MDR-TB) cases (13). The LAMP (loop-mediated isothermal amplification) assay, endorsed by WHO as an alternative to smear microscopy, offers a sensitivity of 95.6–96.6% and comparable specificity, with the advantages of isothermal amplification and field-friendly workflows (14). Line probe assays (LPAs), such as Genotype MTBDRplus and MTBDRsl, facilitate rapid detection of resistance mutations in rpoB, katG, and inhA (for rifampicin and isoniazid), with sensitivity and specificity for MDR-TB detection typically exceeding 90% in high-burden settings, though real-world studies note lower sensitivity for second-line drug resistance (15,16). Micro real-time PCR platforms (e.g., Truenat) have shown diagnostic performance equivalent to Xpert MTB/RIF, providing rapid results within one to two hours and the added benefit of portability in decentralized laboratories (17).

Immunological diagnostics—including antibody detection, antigen detection (such as lipoarabinomannan, LAM), TST, and IGRAs—are increasingly employed for screening and supporting TB diagnosis, though their limitations must be recognized. Serological assays targeting MTB-specific antibodies exhibit poor sensitivity and specificity and are not recommended for clinical use (18). Antigen detection, particularly FujiLAM testing in urine, demonstrates specificity and sensitivity of 93% and 70% in adults and lower values in pediatric populations, with improved yield in HIV-infected patients with low CD4 counts (19). The TST, based on type IV hypersensitivity to purified protein derivative (PPD), yields pooled sensitivity and specificity of 76% and 98%, but performance is influenced by prior BCG vaccination, exposure to non-tuberculous mycobacteria, and host immune status (20,21). IGRAs, such as QuantiFERON-TB and T-SPOT.TB, offer higher specificity (unaffected by BCG) and pooled sensitivity above 95%, with operational advantages in single-visit protocols, but their inability to distinguish active from latent TB and higher costs limit universal applicability (22,23). Novel refinements—such as the T-SPOT TB antigen/PHA ratio and mean spot size measurements—show promise for improving diagnostic discrimination in challenging clinical scenarios, but require further validation (24).

Comparative summary tables (Table 1–3) provide a detailed side-by-side comparison of key diagnostic features, performance metrics, turnaround times, and clinical applicability for microscopy, culture, molecular, and immunological assays. These highlight the trade-offs between speed, accuracy, technical requirements, and suitability for different patient populations and clinical contexts. Despite significant progress, persistent gaps remain. False-negative and false-positive results in microscopy and immunological tests risk misdiagnosis and inappropriate management, while molecular and culture-based methods remain out of reach for many decentralized or resource-limited facilities. The impact of HIV coinfection, pediatric TB, and extrapulmonary disease continues to challenge diagnostic sensitivity, necessitating the integration of complementary technologies. Barriers to the implementation of novel diagnostics—such as cost, infrastructure, and regulatory approval—are particularly acute in high-burden, low- and middle-income countries. No single diagnostic approach is sufficient to address all clinical scenarios, underscoring the need for integrated diagnostic algorithms tailored to local epidemiology and available resources. The reviewed evidence underscores a dynamic landscape of TB diagnostics, with molecular and culture-based methods setting new standards for accuracy, while advanced immunological and rapid point-of-care technologies continue to expand diagnostic reach. The adoption of each diagnostic method should be guided by population needs, disease epidemiology, operational feasibility, and a critical appraisal of local resources and capacity. Ongoing research and implementation studies are necessary to bridge remaining gaps, particularly in the detection of drug resistance, pediatric and extrapulmonary TB, and the deployment of emerging tools in under-resourced settings.

Table 2. Comparison of Major Molecular Diagnostic Methods for Tuberculosis

Feature	Xpert MTB/RIF	LAMP	Line Probe Assay (LPA)	Micro Real-Time PCR (Truenat, etc.)
Principle	Real-time PCR for MTB & rifampicin resistance (rpoB)	Isothermal DNA amplification (16S rRNA, gyrB targets)	PCR amplification + reverse hybridization for resistance gene detection	Real-time PCR for MTB DNA, sometimes sequential drug resistance
Target Genes	rpoB (rifampicin resistance)	16S rRNA, gyrB, others	rpoB, katG, inhA (RIF/INH); gyrA, gyrB, rrs, eis (FLQ/SLID)	IS6110, rpoB, 16S rRNA, others
Sensitivity	98–99.8% (smear+/culture+); ~90% (smear-/culture+)	95.6–96.6%	>90% for MDR-TB; Lower for second-line resistance	92–98% (equivalent to Xpert MTB/RIF)
Specificity	>98%	94–98%	98–100%	>98%
Turnaround Time	~2 hours	~1 hour	5–8 hours	1–2 hours
Drug Resistance	Rifampicin only (INH not detected)	None (MTB detection only)	Rifampicin, Isoniazid, FLQ, SLID, others	Rifampicin, sometimes INH/others
Sample Type	Sputum (also other specimens)	Sputum	Sputum, culture isolates	Sputum, other respiratory samples
Advantages	Rapid; Automated. Minimal hands-on time; Useful for rifampicin resistance screening	Simple, field friendly. No thermal cycler. Low infrastructure	Detects resistance for multiple drugs; Detailed mutation profiling	Portable; Good for decentralized settings; Automated
Limitations	Expensive cartridges. May miss rare rpoB mutations. No INH resistance	Not widely available. Less robust for resistance detection	Needs well-equipped lab. Skilled staff. Lower sensitivity for some drugs	Costly; Specialized equipment; Quality control needed

Table 3. Comparison of Immunological Diagnostic Methods for Tuberculosis

Feature	Antibody Detection	Antigen Detection (LAM/FujiLAM)	Tuberculin Skin Test (TST)	IGRA (QuantiFERON-TB, T-SPOT.TB)	T-SPOT TB Ag/PHA Ratio/Mean Spot Size
Principle	Detects MTB-specific IgG/IgM in blood	Detects MTB antigens (e.g., LAM) in urine	Measures delayed hypersensitivity to PPD	Measures IFN-γ release from T cells in response to MTB antigens	Ratio of TB Ag to PHA, or mean spot size, to improve specificity
Sensitivity	Low–moderate; not reliable	70% adults; 51% pediatrics; higher in HIV+	~76% (pooled); lower in immunosuppressed	>95% (pooled); high in active and latent TB	Under investigation; may improve discrimination

Feature	Antibody Detection	Antigen Detection (LAM/FujiLAM)	Tuberculin Skin Test (TST)	IGRA (QuantiFERON-TB, T-SPOT.TB)	T-SPOT TB Ag/PHA Ratio/Mean Spot Size
Specificity	Low (cross-reactivity, false positives)	93% adults; 87% pediatrics	98% (pooled); affected by BCG, NTM	>98%; not affected by BCG or most NTM	Promising in active/latent TB distinction
Turnaround Time	Few hours	Few hours	48–72 hours	16–24 hours (QuantiFERON), 24–48 hours (T-SPOT)	Variable; requires ELISPOT analyzer
Use Case	Not recommended by WHO	Useful in HIV+ and low CD4 count; screening	Screening; not for active TB diagnosis	Latent TB detection; some role in active TB	Not yet standard; adjunct in research
BCG Interference	Yes	No	Yes	No	No
Advantages	Simple, rapid, low cost	Detects extrapulmonary TB; non-invasive	Field-friendly, inexpensive	Single visit, high specificity	May resolve some IGRA limitations
Limitations	Low accuracy, high false positives	Lower sensitivity in non-HIV/non-severe	False positives in BCG, NTM-exposed	Expensive, requires lab, can't distinguish active/latent	Not fully validated, not widely available

Table 4. Key Strengths and Limitations Across TB Diagnostic Modalities

Modality	Strengths	Limitations
Microscopy	Rapid, low cost, widely available	Low sensitivity, cannot distinguish live/dead bacilli or latent/active TB
Culture	Highest sensitivity and specificity; enables DST	Long turnaround, infrastructure and biosafety needs
Molecular	Fast, detects resistance, good for smear-negative cases	High cost, equipment and maintenance, not all resistance detected
Immunological	Useful for latent TB; high specificity for IGRAs	Cannot distinguish active/latent; influenced by BCG, NTM, immune status

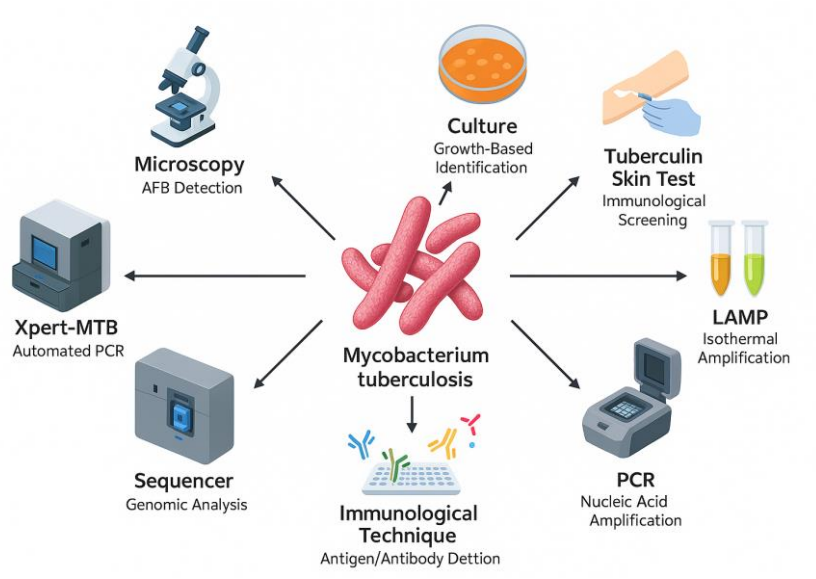

Figure 1 Key diagnostic methods for Mycobacterium tuberculosis.

This infographic presents a central illustration of *Mycobacterium tuberculosis* surrounded by eight diagnostic modalities, each depicted with a modern, vector-style icon and a concise label summarizing its diagnostic principle. The methods featured include microscopy for acid-fast bacilli detection, culture for growth-based identification, tuberculin skin test for immunological screening, LAMP assay for isothermal amplification, PCR for nucleic acid amplification, automated Xpert-MTB for rapid molecular testing, sequencing for genomic analysis, and immunological techniques for antigen or antibody detection. Arrows radiate from the central

bacillus to each diagnostic tool, visually emphasizing the diverse, complementary approaches available for TB detection and characterization in clinical and laboratory settings.

DISCUSSION

This review critically appraises the evolving landscape of *Mycobacterium tuberculosis* diagnostics, emphasizing both the progress and persistent challenges encountered in clinical practice and research. The comparative analysis of traditional and advanced techniques reveals a dynamic field, shaped by emerging technologies that address the longstanding limitations of sensitivity, specificity, and speed in TB detection. Microscopy and culture, though foundational, are limited by low sensitivity in paucibacillary, extrapulmonary, pediatric, and HIV-positive patients, as corroborated by numerous studies reporting suboptimal yields in these groups (1,2). Culture-based drug susceptibility testing remains the reference standard, but delays inherent to MTB's slow growth undermine timely clinical decision-making and increase transmission risk, a finding echoed in global guideline reviews (3). Molecular assays, particularly Xpert MTB/RIF and its Ultra variant, have transformed rapid diagnosis and initial rifampicin resistance screening, with pooled sensitivities and specificities exceeding 90% in most meta-analyses (4,5). However, these platforms do not fully address isoniazid resistance or second-line drug resistance, a limitation also identified in recent systematic reviews and WHO technical updates (6). LAMP and LPAs extend molecular reach, but require further real-world validation, particularly for non-respiratory samples and low-resource settings (7,8). Immunological assays, notably IGRAs, offer high specificity and the operational advantage of single-visit protocols, but their inability to distinguish latent from active infection limits their standalone clinical utility and supports findings from large cohort comparisons (9). The poor performance of serological antibody tests, leading to WHO's formal recommendation against their use, reflects ongoing risks of false-positive and false-negative results (10). Recent progress in mass spectrometry and next-generation sequencing introduces opportunities for comprehensive resistance profiling and ultra-rapid diagnostics; however, these methods are currently constrained by cost, technical expertise requirements, and infrastructure needs, as documented in both multicenter research and expert consensus statements (11,12). Artificial intelligence has shown early promise in automating radiological interpretation and integrating multidimensional diagnostic data, yet its clinical deployment is limited by a lack of large-scale, prospective validation and concerns over algorithm transparency and equity (13-17).

There is a notable paucity of robust comparative data in pediatric, extrapulmonary, and immunocompromised populations—groups that consistently challenge diagnostic paradigms and where test performance is most variable. While this review synthesized data from a wide range of studies, significant heterogeneity exists in study design, population, reference standards, and outcome reporting, limiting direct comparability and generalizability of pooled findings. Additionally, many studies originate from high-resource settings, creating a risk of bias when extrapolating to low- and middle-income countries where TB burden is greatest and operational barriers are most pronounced. The narrative approach, though comprehensive, did not include formal risk-of-bias assessment or meta-analysis, representing a further limitation in the certainty of quantitative estimates presented. Sample size constraints, language restriction to English, and the exclusion of unpublished or non-peer-reviewed data may have led to missed evidence or publication bias. Mechanistically, the reviewed diagnostics each leverage different biological signatures—bacillary burden for microscopy and culture, nucleic acid detection for molecular methods, and host immune response for immunological tests—explaining the observed variance in performance across clinical subgroups and disease states (18-22).

The strengths of this review lie in its breadth of scope, its systematic approach to literature synthesis using a structured framework, and its focus on clinically relevant outcomes and operational realities. By integrating past literature with recent technological advancements, this work highlights both consensus areas—such as the value of molecular diagnostics in high-burden settings—and controversies, such as the optimal algorithm for multidrug-resistant TB or the real-world utility of next-generation sequencing outside research environments. Unexpectedly, several newer diagnostics failed to demonstrate significant improvement in certain challenging cohorts, such as HIV co-infected or pediatric patients, reinforcing the need for targeted innovation and implementation science in these populations. Clinical relevance is further underscored by the increasing threat of drug resistance and the operational need for rapid, decentralized testing that can be feasibly deployed in resource-limited settings (7, 18).

Future research should focus on large-scale, prospective head-to-head evaluations of diagnostic algorithms in diverse real-world populations, including children, individuals with HIV, and those with extrapulmonary disease. Studies are needed to evaluate cost-effectiveness, workflow integration, and impact on clinical outcomes, rather than diagnostic accuracy alone. It is equally critical to address implementation challenges such as training, supply chain management, regulatory approval, and equitable access. Investments in digital infrastructure and AI validation, combined with operational research in low-resource settings, will be necessary to close the persistent diagnostic gap and realize the full promise of emerging technologies. In summary, while substantial progress has been made, there is a continued need for innovation, rigorous validation, and equitable implementation of advanced TB diagnostics, particularly for populations and settings most affected by the global TB epidemic.

CONCLUSION

This review demonstrates that while recent advancements in mass spectrometry, artificial intelligence, and genomics have significantly expanded the diagnostic repertoire for *Mycobacterium tuberculosis*, substantial gaps persist in the sensitivity, specificity, and accessibility of these technologies, especially in high-burden, resource-limited settings. The integration of these novel modalities with established methods offers promise for more rapid and precise TB detection and drug resistance profiling, but widespread clinical adoption is constrained by cost, technical complexity, and lack of robust validation across diverse populations. Actionable recommendations include the prioritization of head-to-head trials in real-world contexts, targeted innovation for vulnerable groups, and investment in scalable, context-appropriate diagnostic solutions. Ultimately, the future of TB diagnostics hinges on a multidisciplinary, patient-centered approach that bridges technological innovation with public health impact.

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