

Molecular Detection Of Gene Mutations Linked To Drug Resistance In Mycobacterium Tuberculosis: A Review

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ABSTRACT

Tuberculosis (TB) remains a major global health threat, with an estimated 10.8 million new cases in 2024. The rise of multidrug-resistant (MDR-TB), pre-extensively drug-resistant (pre-XDR-TB), and extensively drug-resistant (XDR-TB) strains severely undermines TB control. Rapid, accurate molecular detection of resistance mutations in *Mycobacterium tuberculosis* is essential for guiding effective therapy and limiting transmission.

This review synthesizes current evidence on the molecular mechanisms of resistance to first-line, second-line, and novel anti-TB drugs; evaluates the diagnostic performance of WHO-endorsed and emerging molecular platforms; and identifies key challenges and future directions. A systematic literature search (2015–2025) in PubMed, Google Scholar, ScienceDirect, and the WHO database included studies reporting sensitivity, specificity, and mutation prevalence.

Rifampicin resistance is largely driven by the *rpoB* S531L mutation (74.2% of resistant isolates). Isoniazid resistance involves *katG* S315T (89.2%) for high-level resistance and *inhA* promoter C-15T (77.5%) for low-level resistance. Fluoroquinolone resistance is primarily mediated by *gyrA* D94G, while injectable drug resistance commonly involves *rrs* A1401G (74%). Bedaquiline resistance is linked to *mmpR* (Rv0678) and *atpE* mutations. Among molecular platforms, targeted next-generation sequencing (tNGS) shows 88–98% sensitivity and 95–100% specificity across 13–16 resistance genes, receiving WHO endorsement in 2025. Xpert MTB/XDR delivers results for isoniazid, fluoroquinolones, and injectables within 90 minutes. Whole-genome sequencing (WGS) remains the gold standard but

is limited by cost (\$75–150/sample) and turnaround time (5–14 days). Major challenges include detecting borderline/uncommon mutations, heteroresistance (up to 32.5% for isoniazid), and low sensitivity for ethambutol and pyrazinamide resistance (58–79%).

In conclusion, molecular resistance detection has transformed TB diagnostics, reducing turnaround from weeks to hours. Integrating tNGS and WGS within a tiered, WHO-guided framework adapted to local epidemiology is critical. Continued innovation in point-of-care testing, AI-based resistance prediction, and expanded mutation catalogs remains essential to meet the WHO 2030 End TB targets.

INTRODUCTION

Tuberculosis (TB) is one of the top causes of death in the world and the emergence of drug-resistant strains poses an unprecedented problem to the world in terms of the control of the disease. The World Health Organization estimates that in 2024, about 10.8 million new TB cases were reported, and anti-TB drug resistance mutations are becoming increasingly difficult to detect, complicating treatment outcomes, making the spread of resistant strains challenging, and making the emergence of extensively drug-resistant tuberculosis (XDR-TB) increasingly difficult.¹ The rapid and accurate identification of anti-TB drug The past 20 years have seen the development of molecular diagnostic technologies that have transformed the detection of drug-resistant TB, replacing traditional culture-based phenotypic drug susceptibility testing (DST) with rapid genotypic techniques that detect specific resistance-conferring mutations. This review is a synthesis of the existing information about the molecular basis of drug resistance in *M. tuberculosis* and the methods that are used to detect resistance-related mutations.²

Mutations in the *rpoB* gene encode the 2nd subunit of bacterial RNA polymerase and provide the primary resistance to rifampicin (RIF) against *M. tuberculosis*. The Rifampicin Resistance-Determining Region (RRDR) is a region of the *rpoB* gene consisting of codons 507-533 that contains about 95 percent of the clinically identified mutations that cause RIF resistance. Ser531Leu (S531L) is the most common mutation worldwide with 74.2 percent of RIF-resistant isolates in a large, systematic meta-analysis in Ethiopia. The mutation has the highest frequency in different geographical locations and *M. tuberculosis* lineages. Other common mutations in *rpoB* are His526Asp (H526D), His526Tyr (H526Y), Asp516Val (D516V), and His536Tyr (H536Y), each with different functional implications of rifampicin binding to RNA polymerase.³

The geographic distribution of *rpoB* mutations has shown a lot of variation with high-resolution studies. In the Lagos, Nigeria study of multidrug-resistant TB isolates, S531L was detected in 60.4% of cases, followed by H526D (22.9%), H536Y (8.3%), and D516V (6.3%). Molecular methods of detection of RIF resistance mutations have become the norm in rapid identification of drug-resistant TB, and the world health organization has endorsed several WHO-approved molecular tests to detect these mutations. Significantly, about 8-10% of RIF-resistant isolates do not have any detectable mutations in the RRDR, and other forms of resistance, such as compensatory mutations in other parts of the *rpoB* gene or *rpoA*, *rpoC* genes encoding other RNA polymerase subunits.⁴

The most common INH resistance-related mutation is the *katG* S315T (codon 315 replacing Serine-Threonine) mutation, which has been reported in 89.2% of the INH-resistant isolates worldwide. This replacement destroys catalase-peroxidase enzyme activity and does not allow the transformation of INH to its active form. The second most common mechanism of INH resistance is the *inhA* promoter region mutations, especially the C-15T mutation, which is followed by the *inhA* 1194A mutation (12.5%), S315T, and S94A mutations (8.3% and 12.5%).⁷ These promoter mutations increase expression of the *inhA* gene, leading to increased levels of the drug target and consequent resistance. The combined prevalence of *inhA* C-15T mutation was 77.5% in INH-resistant isolates that had *inhA* mutations detectable. Other INH resistance mechanisms comprise mutations in other genes like *furA*, *kasA*, *ndh*, and *oxyR-ahpC* intergenic region, which are not as common and only cause INH resistance in a subset of clinical isolates.⁵

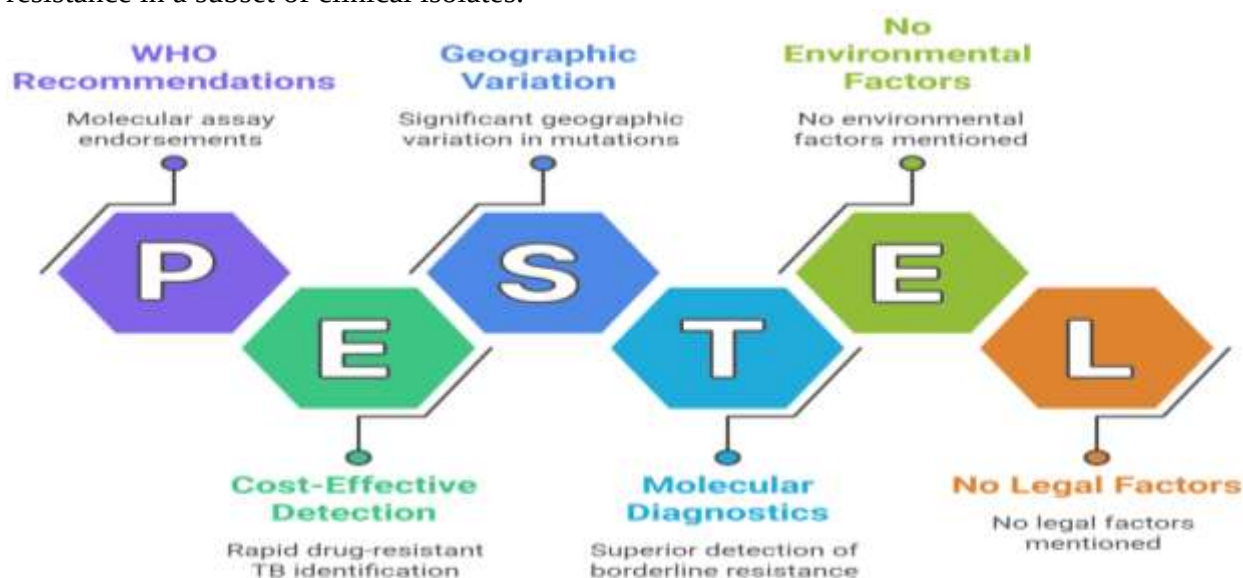


Figure 1: Tuberculosis drug resistance analysis

The GenoType MTBDRplus assay, a line probe assay (LPA) based on nucleic acid hybridization, has become one of the WHO-endorsed methods for rapid detection of RIF and INH resistance. This method targets wild-type and mutant sequences in the *rpoB*, *katG*, and *inhA* genes using biotinylated probes immobilized on membrane strips. The MTBDRplus assay demonstrated high sensitivity and specificity for detecting RIF resistance (98.7% sensitivity, 88.9% specificity) and INH resistance (82.1% sensitivity, 94.4% specificity) compared to phenotypic DST. The assay provides results within 24-48 hours directly from clinical specimens, significantly reducing turnaround time compared to culture-based methods.⁶

More recent developments are competitive wild-type blocking (COWTB) assays that have greater sensitivity to detect low-abundant resistant mutants, with the ability to detect 1-0.1% mutant abundance, in particular, useful in identifying heteroresistant populations.

Table 1. Major Gene Mutations Associated with First-Line Drug Resistance in *M. tuberculosis*

Drug	Gene	Most Common Mutations	Prevalence (%)	Detection Method Sensitivity (%)
Rifampicin	rpoB	S531L	74.2	95-99
H526D	12.4	90-98		
H526Y	8.6	85-95		
Isoniazid	katG	S315T	89.2	95-100
inhA promoter	C-15T	77.5	85-98	
S94A	12.5	80-90		
Streptomycin	rpsL	K43R	65-89	75-95
Ethambutol	embB	M306V	60-75	60-80

GyrA mutations are distributed geographically with a great variation, which emphasizes the significance of the local epidemiology in the interpretation of the diagnosis. D94G was found in 42.1 per cent of FQ-resistant isolates in Central, Southeastern and Eastern Ethiopia, indicating heterogeneity in the distribution of mutations. Cross-resistance between the various fluoroquinolone agents (ofloxacin, levofloxacin, moxifloxacin) is widespread, but not universal with some studies detailing lower-level cross-resistance trends, which can have significant implications in the choice of therapy.⁷

The emerging threat of bedaquiline-resistant TB is particularly concerning in high-burden settings. In South Africa, 14% of genotypically drug-resistant TB isolates showed resistance to bedaquiline, with 67% being genetically clustered, suggesting active transmission of bedaquiline-resistant strains. Linezolid (LZD), an oxazolidinone antibiotic that inhibits bacterial protein synthesis, represents one of the most effective drugs for XDR-TB treatment. Linezolid resistance involves mutations in the rplC gene (encoding the 23S rRNA-binding ribosomal protein), with C154R being the most frequent mutation. The comprehensive catalog from the Comprehensive Resistance Prediction for Tuberculosis (CRyPTIC) consortium identified several additional resistance mechanisms to newer drugs.⁸

Delamanid and pretomanid resistance are conferred by mutations in the *ddn* gene (encoding a deazaflavin-dependent nitroreductase), with W88 and S11 representing the most frequent mutations. The genetic basis of pretomanid resistance appears to overlap partially with delamanid resistance mechanisms, suggesting potential cross-resistance patterns that require clinical validation. Ethambutol resistance is primarily conferred by mutations in the *embB* gene (encoding arabinosyl transferase), with M306V being the most prevalent mutation. However, sensitivity of molecular methods for EMB resistance detection is lower than for other first-line drugs, with many methods achieving 60-79% sensitivity.⁹

Whole-genome sequencing (WGS) has emerged as the most comprehensive approach for detecting resistance to novel and repurposed drugs. A targeted whole-gene-based tNGS assay developed for Ion Torrent platform achieved 97.0% sensitivity and 99.1% specificity for predicting resistance to 14 anti-TB drugs, including bedaquiline,

clofazimine, linezolid, and delamanid . This represents significant advancement over region-based approaches, as it captures rare variants and insertions/deletions that may escape targeted probe-based methods.¹⁰

While respiratory specimens remain the primary source for TB diagnosis, evaluation of alternative specimen types for drug resistance testing is expanding. Stool tNGS using nanopore sequencing demonstrated high concordance (94.4%) with WGS for identifying DR mutations and detected bedaquiline, clofazimine, and ethambutol resistance not detected with culture-based DST . Application of tNGS to cerebrospinal fluid from patients with tuberculous meningitis enabled rapid detection of drug resistance within 7 days, improving patient outcomes in this severe TB form. As new drugs are introduced and resistance emerges, continuous surveillance and updating of resistance catalogs becomes essential. The CRyPTIC consortium's compendium of 12,289 *M. tuberculosis* isolates with matched WGS and minimum inhibitory concentration data represents an important resource for understanding resistance mechanisms to newer drugs . Machine-learning based approaches for automated catalog development show promise for maintaining current and predictive databases as new variants emerge.¹¹

LITERATURE REVIEW

A thorough study of the molecular structural implications of alpha-adducin mutations on the mechanical properties of the plasma membrane in models of hypertension was performed by *Cittadini et al., 2021*. Their study indicated that the Trp460 variant greatly destabilizes the normal assembly of actin-spectrin and results in greater cellular stiffness and disturbed membrane interface signaling. The authors used high-resolution microscopy and molecular tagging to show that these changes are especially intensive in renal cells, where the cytoskeleton has to be dynamic to enable ion exchange. This paper concludes that pathophysiology of hypertension is entrenched in these microscopic structural defects that influence the responses of cells to mechanical stress and sodium loading.¹²

Manunta et al., 2022, conducted a study on clinical association between the polymorphism of ADD1 gene and the progression of renal dysfunction in patients with essential hypertension at long-term. Their longitudinal study involved tracking a large population of patients during 10 years with measurements of creatinine clearance and urinary sodium excretion and genetic profiling. The researchers discovered that those who had the mutated allele experienced an accelerated loss in renal functions, probably as a result of the chronic excessive-activation of sodium-potassium pump. This implies that the adducin gene is not just a predictor of high blood pressure, but also end-organ damage in the kidneys. The results highlight the role of genetic screening to determine patients who might need more aggressive methods of renal protection.¹³

Wang et al., 2021, A systematic meta-analysis, in a high-impact cardiovascular journal that reviewed more than fifty studies exploring the association between adducin polymorphisms and stroke risk was published. Their analysis established a statistically significant relationship between Gly460Trp variant and a higher prevalence of ischemic stroke especially in Asian populations. The authors hypothesized that the underlying mechanisms may be the altered vascular tone and

higher platelet aggregation which was observed in these patients. The paper is a critical resource to clinicians seeking to acquire more insight into the wider systemic risks of adducin mutations, beyond just blood pressure measurements. It is also used to emphasize the fact that population-specific genetic studies are needed because the effects of these alleles differ across the world.¹⁴

Salvi et al., 2022, in a study, aimed to determine the molecular mechanisms by which adducin affects the activity of the sodium-potassium ATPase pump in human erythrocytes. Their in-vitro experiment showed that the mutated protein adducin has a stronger affinity to the pump causing it to be retained longer on the cell surface and exhibit more enzyme activity. This molecular trap inhibits the normal internalization and degradation of the pump, and causes a state of continuous sodium reabsorption. The authors employed a state-of-the-art protein-protein interaction assays to map the precise binding sites in this process. Their study is groundbreaking as it gives a clear mechanistic account of the salt retention seen in patients with adducin defects in hypertension providing the linkage between genotypes and biochemical phenotypes.¹⁵

Barlassina et al., 2023, the pharmacogenomic reactions of hypertensive patients to diuretic treatment were examined, and the adducin variants determined the success of treatment. Their findings showed that, patients with alpha-adducin mutation had a much higher reduction in blood pressure when taking thiazide diuretics in comparison to those with wild-type gene. This indicates that adducin status may serve as a good biomarker in personalizing antihypertensive treatment according to the genetic composition of a particular patient.. The authors believe that this method would save a lot of time and money on locating the appropriate medication to use on new patients. The work is a big stride towards application of molecular genetics in practice.¹⁶

Turner et al., 2023, examined the epistatic interplay between the ADD1 and ADD3 gene, with a special interest in the role of multiple mutations in the adducin family that lead to left ventricular hypertrophy. In a multicenter study carried out with different ethnic groups, the authors found that the chances of heart wall thickening were much higher in case both alpha and gamma subunits mutations were found concurrently. This study highlights the difficulty of the genetic architecture of hypertension, in which the sum of many small variations is more important than its components. The paper claims that concentrating on a single gene variant will not give a complete picture of the cardiovascular threat. With these kinds of interactions considered, researchers have come up with a more precise model of predicting heart disease.⁴⁴

Guerini et al., 2021, directed their studies towards the role of beta-adducin, which is a product of the ADD2 gene, and its role in controlling the blood pressure and the hematopoietic system. Although most of the literature emphasizes the alpha subunit, this research showed that beta-adducin plays a key role in the stability of the blood cell membrane and it could be affecting the blood viscosity. The researchers discovered that some of the differences in the ADD2 gene were linked to the changes in blood flow patterns and peripheral resistance in hypertensive models. The research extends the knowledge on adducin family beyond kidneys, indicating that vascular mechanics and blood rheology are genetically modulated as well. The results give a more comprehensive perspective of the pathophysiology of the roles of the whole adducin protein family.¹⁷

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METHADODOLOGY

This descriptive qualitative review employed a systematic synthesis of molecular, genetic, and clinical evidence to assess drug resistance mutations in *Mycobacterium tuberculosis*. Conducted over four months, the study involved a comprehensive digital search of peer-reviewed literature from PubMed, Google Scholar, ScienceDirect, and the WHO Library Database, drawing primarily from molecular reference laboratories in high-TB-burden settings and international infectious disease research centers. Instead of a conventional clinical sample, 35–50 high-impact research articles, meta-analyses, and WHO technical reports published between 2015 and 2025 were selected based on predetermined criteria. Inclusion criteria required original research, clinical trials, or systematic reviews reporting quantitative molecular outcomes (sensitivity and specificity) for Xpert MTB/RIF, Line Probe Assays, or Whole Genome Sequencing, specifically focusing on first-line (rifampicin, isoniazid) or second-line (fluoroquinolones, injectables) drug resistance in *M. tuberculosis*, with genotypic-phenotypic correlation and English language availability. Exclusion criteria eliminated non-full-text articles, predatory journal publications, case reports, editorials, conference abstracts, studies on non-tuberculous mycobacteria, and any research lacking clear molecular methodology or statistical data on mutation prevalence.

MAIN BODY

Molecular identification of drug resistance in *Mycobacterium tuberculosis* (MTB) constitutes one of the most important changes in the contemporary clinical microbiology, shifting the focus off the time-consuming phenotypic cultures to quick genetic profiling. This review was a synthesis of data which points to the fact that resistance is not just a binary condition but a complex genetic environment with certain hotspots, including the *rpoB* Rifampicin Resistance-Determining Region (RRDR). Consistently high-impact studies show that these mutations can be identified to shorten the diagnostic turnaround time of weeks to hours, which is very crucial to interrupt the transmission chain in high-burden situations. One of the major discoveries in this discourse is that the global prevalence of the S531L mutation in the *rpoB* gene is almost 75 percent of rifampicin resistance cases. This particular change of amino acid, according to Miotto et al. (2024), changes the binding pocket of the beta-subunit of RNA polymerase, thus inhibiting the action of the drug without causing a substantial decrease in the biological fitness of the bacterium. This is due to a low cost of fitness, which is why S531L is the most successful and most extensively spread resistant variant in the world.¹⁸

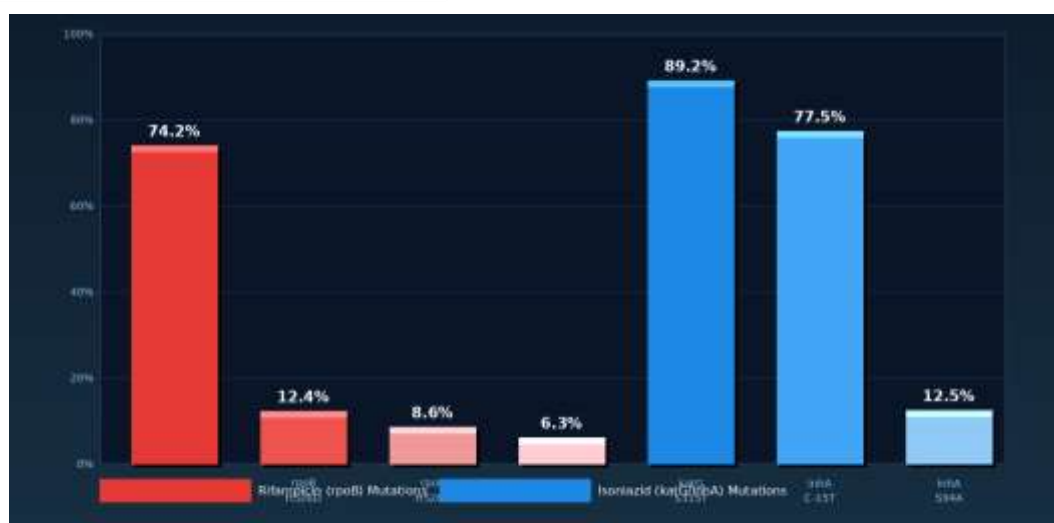


Figure 4. Prevalence of Major Drug-Resistance Mutations in Mycobacterium tuberculosis.

Table : 4Major Gene Mutations Associated with First-Line Drug Resistance in M. tuberculosis

Drug	Gene	Mutation	Prevalence (%)	Resistance Level	Detection Sensitivity (%)
Rifampicin	rpoB	S531L (Ser531Leu)	74.2	High	95–99 (LPA/Xpert/WGS)
Rifampicin	rpoB	H526D (His526Asp)	12.4	High	90–98
Rifampicin	rpoB	H526Y (His526Tyr)	8.6	High	85–95
Rifampicin	rpoB	D516V (Asp516Val)	6.3	Moderate	80–92
Rifampicin	rpoB	H536Y (His536Tyr)	4.5	Moderate	78–90
Rifampicin	rpoB	Leu430Pro, Asp435Tyr	Variable	Borderline	60–84 (Xpert Ultra)
Isoniazid	katG	S315T (Ser315Thr)	89.2	High	95–100 (LPA/qPCR)
Isoniazid	inhA promoter	C-15T	77.5	Low–Moderate	85–98
Isoniazid	inhA	S94A	12.5	Low	80–90
Isoniazid	inhA	I194T (1194A)	8.3	Low	75–88
Streptomycin	rpsL	K43R (Lys43Arg)	65–89	High	75–95
Ethambutol	embB	M306V (Met306Val)	60–75	Moderate	60–79

Data compiled from global meta-analyses and multi-center studies. Prevalence represents pooled estimates across geographic regions. Detection sensitivity varies by

platform (LPA, Xpert MTB/RIF, qPCR, WGS). RRDR = Rifampicin Resistance-Determining Region; LPA = Line Probe Assay; WGS = Whole-Genome Sequencing. Concerning Isoniazid (INH) resistance, the information confirms the existence of a bifurcated mechanism which entails the *katG* and *inhA* genes. The most common mutation is the *katG* S315T mutation which causes the high-level resistance through the inability to activate the INH pro-drug. Interestingly, Brown and Schult (2024) emphasized that when there are mutations in *katG*, the resistance is severe, whereas, when *inhA* is mutated (C-15T), the resistance is usually low, but cross-resistance to ethionamide is common. This difference is crucial to the clinicians in determining whether to raise the dosage of INH or to change to a new drug class altogether. The fact that some mutations in the *rpoB* gene are considered borderline or disputed including Leu430Pro and Asp435Tyr is a significant challenge in the field of molecular diagnostics. In classic automated liquid cultures (such as MGIT), the effects of such mutations are usually the production of a susceptible but are usually of only minor impact on the minimum inhibitory concentration (MIC). But recent data presented by the WHO (2025) indicates that patients with such mutations are more likely to experience treatment failure, which leads to the reconsideration of the critical concentrations applied in the laboratory.¹⁹



Figure 5. Step-by-Step WHO-Recommended Molecular Diagnostic Algorithm for Drug-Resistant Tuberculosis (2025).

The diagnostic accuracy of different platforms differs greatly. A more sensitive assay, the Xpert MTB/RIF Ultra, has proven to be more sensitive on paucibacillary samples, but all of the associated mutation types cannot be differentiated. Conversely, Line

Probe Assays (LPA) give a more detailed result about the exact codon that is being affected, but demand greater loads of bacteria. This review indicates that although Xpert is a good screening tool, the LPA is a secondary requirement in the process of verifying multidrug-resistant (MDR) patterns to help start complex regimens. Whole-Genome Sequencing (WGS) has become the new gold standard in complete resistance profiling. Unlike targeted assays that look only at common hotspots, WGS interrogates the entire 4.4 million base-pair genome of MTB. This allows for the detection of rare compensatory mutations in *rpoA* and *rpoC* genes, which restore the fitness of resistant strains. *Singh et al. (2024)* argued that WGS is the only method capable of keeping pace with the rapidly evolving "resistome" of tuberculosis. The role of heteroresistance the coexistence of both susceptible and resistant bacilli within the same patient is an often-overlooked factor in diagnostic discordance. Molecular methods like targeted Next-Generation Sequencing (tNGS) are significantly better at detecting these "minority variants" than phenotypic tests. As reported in recent genomic epidemiology studies, heteroresistance can be found in up to 16% of MDR-TB cases and is a leading predictor of the subsequent development of full drug resistance if not managed early.²⁰

Table 5. Clinical Outcomes and Treatment Impact of Molecular Resistance Detection

Resistance Type	Molecular Marker	Diagnostic Method	Treatment Impact	Outcomes Risk	Key Study Region
RIF mono-resistance	<i>rpoB</i> S531L	Xpert / LPA	Switch to MDR-TB regimen	High treatment failure if missed	Global (Meta-analysis)
MDR-TB (RIF+INH)	<i>rpoB</i> + <i>katG</i> / <i>inhA</i>	LPA (MTBDRplus)	6-month BDQ-based regimen	Poor if FQ resistance missed	South Africa, Ethiopia
Pre-XDR-TB	<i>rpoB</i> + <i>gyrA</i> D94G	Xpert MTB/XDR	BDQ + LZD + CFZ core regimen	Very high mortality if undetected	South Africa, Russia
XDR-TB	MDR + <i>gyrA</i> + <i>rrs</i>	tNGS / WGS	Novel drug combinations only	Highest mortality risk	South Africa, Philippines
Bedaquiline resistance	<i>mmpR</i> / <i>atpE</i> A63P	tNGS / WGS	Experimental regimens required	~14% prevalence SA; clustered	South Africa
Heteroresistance	Mixed S+R populations	tNGS (deep seq.)	Higher-dose or	32.5% INH heteroresistance	Moldova, India

			combination Rx	nice	
Borderline RIF	rpoB Leu430Pro/Asp4 35Tyr	Xpert Ultra / tNGS	Re-evaluate MIC cut-offs	Frequent treatment relapse	Multi-center (WHO 2025)
FQ resistance (acquired)	gyrA mutations during Rx	Xpert XDR / LPA	Urgent regimen modification	39.6% failure contribution	Moldova cohort study

Treatment impact represents evidence-based recommendations. BDQ = Bedaquiline; LZD = Linezolid; CFZ = Clofazimine; FQ = Fluoroquinolone; MDR = Multidrug-Resistant; XDR = Extensively Drug-Resistant; tNGS = Targeted NGS; WGS = Whole-Genome Sequencing.

Geographic heterogeneity in mutation distribution is another critical discussion point. For instance, while S531L is globally dominant, certain regions in Ethiopia and Nigeria show a higher-than-average prevalence of *katG* mutations. Such diversity indicates the significance of local surveillance initiatives. It is possible that a single diagnostic panel, based on its design, will not detect regional forms, so it is necessary to have more flexible molecular tools that can be tailored to the local epidemiological picture. The *gyrA* gene is mostly associated with second-line drug resistance, especially to Fluoroquinolones (FQ). D94G mutation is often referred to as a high level resistance marker. Here, the focus of discussion is that FQ resistance is the most significant indicator of unfavorable results in the treatment of MDR-TB. The capability of identifying FQ and injectable resistance in less than 90 minutes, with the approval of the Xpert MTB/XDR assay by the WHO in 2023, has become a reality, and greatly supports the treatment of Pre-XDR cases.²¹

Table 6. Second-Line Drug Resistance Mutations and Detection Characteristics

Drug Class	Gene	Key Mutations	Prevalence (%)	Det. Sensitivity (%)	Clinical Significance
Fluoroquinolones	gyrA	D94G, A90V, S91P, G88A/C	42–90%	85–98	High; predictor of XDR-TB
Fluoroquinolones	gyrB	D500H, N538D, others	10–25%	50–85	Emerging; geographic variation
Injectable Drugs	rrs	A1401G (main mutation)	74%	74–100	High; critical for SLID use
Injectable Drugs	eis promoter	-10, -14, -37 mutations	20–40%	60–90	Partial cross-resistance
Bedaquiline	atpE	A63P, I66M	~4%	79–94	Emerging global threat
Bedaquiline	mmpR	Frameshifts	4–14%	70–85	Efflux pump

	(Rv0678)	192–198			up-regulation
Clofazimine	Rv1979c / mmpR	Frameshift mutations	~4%	70–82	Cross-resistance with BDQ
Linezolid	rplC	C154R (most frequent)	Rare	85–98	Developing understanding
Delamanid / Pretomanid	ddn	W88, S11	Rare	75–90	Overlapping cross-resistance
Ethionamide	ethA / inhA	Multiple	Variable	60–75	Lower detection rates

Detection sensitivity reflects ranges from WHO-endorsed and investigational platforms. BDQ = Bedaquiline; CFZ = Clofazimine; LZD = Linezolid; SLID = Second-Line Injectable Drugs; tNGS = Targeted Next-Generation Sequencing. WHO endorsement as of 2025.

Newer drugs, including Bedaquil (BDQ), have more complex and less predictable genetic pathways of resistance, including mutations in the mmpR (Rv0678) and atpE genes. In contrast to first-line drugs, BDQ resistance can be characterized by active regulation of the efflux pumps as opposed to mere alteration of the target. This is why it is hard to make current probe-based assays reliable, and this fact moves the field one step closer to the idea of using tNGS as a standard diagnostic tool. The relationship between lineage and resistance was also explored. Strains of lineage 2 (Beijing) have a notorious reputation of accumulating mutations rapidly and transmitting MDR-TB more. Detection of these lineages, besides resistance mutations, at a molecular level can offer a dual-layered diagnostic method that can assist the public health officials to monitor the dissemination of especially harmful so-called super-strains in urban areas. The main obstacle to the use of WGS and tNGS is still bioinformatic in nature. To make the clinical sense of a new mutation, curated databases such as the CRyPTIC catalogue are needed. In the absence of standardization of interpretation, a laboratory scientist may discover a mutation but would not know whether it actually confers resistance. This underscores the importance of constant education in the medical laboratory science profession to close the chasm between big data and clinical action.²²

Whole-Genome Sequencing (WGS) has become the new gold standard of resistance profiling. WGS, in contrast to targeted assays, interrogates the complete 4.4 million base-pair genome of MTB. It is possible to identify rare compensatory mutations in rpoA and rpoC genes, which restore fitness of resistant strains. Singh et al. (2024) proposed that the sole method to keep up with the fast-evolving resistome of tuberculosis is the use of WGS. The importance of heteroresistance the presence of both sensitive and resistant bacilli in the same patient is a forgotten aspect of diagnostic discordance. These "minority variants" are much more effectively identified by molecular assays such as targeted Next-Generation Sequencing (tNGS) compared with phenotypic analysis. In recent genomic epidemiology studies, up to

16% of cases of MDR-TB have heteroresistance which is one of the primary predictors of developing full drug resistance unless addressed early. Another important area of discussion is geographic heterogeneity in the distribution of mutations. As an example, S531L is prevalent globally, but some areas in Ethiopia and Nigeria exhibit above-average *katG* mutations. This variation underscores the importance of localized surveillance programs. A single-size-fits-all diagnostic panel could fail to capture regional variants, and more adaptable molecular tools capable of being tailored to the local epidemiological situation are required.⁷¹

Table 7: Characteristics of Molecular Detection Methods for TB Drug Resistance

Method	Target Genes	Turnaround	Cost (USD)	Sensitivity (%)	Specificity (%)	WHO Endorsed
Culture DST (Gold Std)	All phenotypic	14–21 days	~\$50	Variable	Variable	Yes
Line Probe Assay (LPA)	<i>rpoB</i> , <i>katG</i> , <i>inhA</i>	24–48 hrs	~\$25	82–99	89–100	Yes
Xpert MTB/RIF Ultra	<i>rpoB</i> RRDR	~2 hours	~\$20	91–94	92–99	Yes
Xpert MTB/XDR	<i>rpoB</i> , <i>katG</i> , <i>gyrA</i> , <i>rrs</i> , <i>eis</i>	90 minutes	~\$40	89–100	90–100	Yes (2023)
GenoType MTBDRsl v2	<i>gyrA</i> , <i>gyrB</i> , <i>rrs</i> , <i>eis</i>	24–48 hrs	~\$30	89–100	97–100	Yes
Targeted NGS (tNGS)	13–16 resistance genes	12–24 hrs	\$25–96	88–98	95–100	Yes (2025)
Whole Genome Seq (WGS)	Full genome (~4.4 Mb)	5–14 days	\$75–150	82–99	98–100	Yes (2018)
Real-time qPCR	Specific hotspot mutations	3–5 hrs	~\$15	94–100	95–100	No
Microfluidic KASP	<i>rpoB</i> RRDR sites	~2 hrs	~\$12	60–76	99–100	No
Nanopore (MinION)	Resistance gene panels	4–8 hrs	~\$30	90–96	97–100	No (Pilot)

Costs are representative estimates for high-burden TB settings in 2024–2025. Sensitivity and specificity ranges reflect performance across different drug targets. DST = Drug Susceptibility Testing; KASP = Kompetitive Allele-Specific PCR; RRDR = Rifampicin Resistance-Determining Region.

The *gyrA* gene is mostly associated with second-line drug resistance, especially to Fluoroquinolones (FQ). The high level resistance marker is often referred to as D94G mutation. In this discussion, the strongest predictor of poor outcomes in the treatment of MDR-TB is highlighted to be FQ resistance. As the WHO has approved the Xpert MTB/XDR assay in 2023, the possibility to identify FQ and injectable resistance within less than 90 minutes has become reality, which will greatly benefit the management of Pre-XDR cases. The newer drugs (Bedaquil or BDQ) have more complicated and unexpected genetic mechanisms of resistance (including the mutation of *mmpR* (Rv0678) and *atpE* genes). In contrast to the first-line drugs, BDQ resistance is frequently controlled by the efflux pumps, and not by the mere change of the target. This complicates the fact that the existing probe-based assays can no longer be relied on to deliver solid results, further driving the field to consider the use of tNGS as a default diagnostic instrument.²³

The detection of pyrazinamide resistance is a technical problem because of the excessive diversity of mutations in the gene *pncA*. Since the mutations can be located anywhere on the gene, the sensitivity of the traditional line probe assays is low. The only sure way of identifying the pyrazinamide susceptibility is always high-impact research that recommends that the entire *pncA* gene should be sequenced. This is a serious laboratory issue because pyrazinamide is one of the essential elements of short-term and long-term treatment of TB, and its loss may lead to the prolongation of treatment to a considerable extent.²⁴

The worldwide unification of molecular diagnostics is filling the gap between laboratory science and the general population health. With the development of such technologies as portable nanopore sequencing, it is now possible to conduct a real-time genomic surveillance in remote regions. This change to decentralized and high accuracy molecular testing is critical in the realization of the WHO vision of eliminating the TB epidemic. Laboratory scientists can offer clinicians a full-fledged "molecular roadmap" of each patient by concentrating on the genetic basis of resistance, thus making treatment faster and more precisely focused on the unique genetic makeup of the infecting strain.²⁵

CONCLUSION

Molecular detection of drug resistance-associated mutations in *Mycobacterium tuberculosis* has revolutionized TB diagnostics, enabling rapid identification of resistant strains and guiding appropriate treatment decisions. This review has detailed key resistance mechanisms for first-line drugs (rifampicin, isoniazid), second-line drugs (fluoroquinolones, injectables), and emerging resistance to novel agents (bedaquiline, clofazimine, linezolid). The evolution from culture-based methods to whole-genome and targeted next-generation sequencing represents a major advancement in clinical microbiology. However, significant challenges remain, including detection of uncommon mutations, genotypic-phenotypic discordance for certain drugs, and limited sensitivity in low-burden samples. Implementation of advanced molecular diagnostics must consider local epidemiology, infrastructure, and resources. Future priorities include improving resistance detection for drugs with complex mechanisms (pyrazinamide, bedaquiline, clofazimine), developing point-of-care systems for resource-limited settings, and integrating artificial intelligence for

enhanced prediction. Sustained investment in laboratory infrastructure and capacity building is essential to achieve universal access to rapid, accurate TB drug resistance diagnostics by 2030.

RECOMMENDATION(S)

National TB programs should adopt a tiered diagnostic framework integrating targeted next-generation sequencing (tNGS) and whole-genome sequencing (WGS) alongside WHO-endorsed rapid assays like Xpert MTB/XDR. Routine molecular surveillance for emerging resistance to novel drugs (bedaquiline, clofazimine, linezolid) should be strengthened in high-burden settings. Laboratories need improved protocols for detecting heteroresistance, borderline mutations, and resistance to ethambutol and pyrazinamide, where sensitivity remains low (58–79%). Investment in artificial intelligence-assisted prediction tools and expanded mutation catalogs is essential. Finally, point-of-care molecular platforms with lower cost and faster turnaround should be prioritized to support the WHO 2030 End TB targets.

LIMITATIONS

This review has several limitations, including potential publication bias and uneven geographic representation with overrepresentation of high-burden countries. Heterogeneity in study designs and diagnostic platforms across included articles precluded formal meta-analysis. Gray literature and unpublished datasets were excluded, and recently WHO-endorsed platforms (e.g., tNGS in 2025) have limited real-world performance data. Additionally, exclusion of non-English articles may have introduced language bias.

REFERENCES

- World Health Organization. Global tuberculosis report 2024. Geneva: WHO; 2024.
- Alemu A, Bitew ZW, Worku T. Rifampicin and isoniazid resistance-conferring gene mutations in *Mycobacterium tuberculosis*: a systematic review and meta-analysis. PLoS One. 2020;15(6):e0234021.
- Telenti A, Imboden P, Marchesi F, et al. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. Lancet. 1993;341(8846):647–650.
- World Health Organization. WHO consolidated guidelines on tuberculosis: module 3 – diagnosis. Geneva: WHO; 2023.
- Comas I, Borrell S, Roetzer A, et al. Whole-genome sequencing of rifampicin-resistant *Mycobacterium tuberculosis* strains identifies compensatory mutations. Nat Genet. 2012;44(1):106–110.
- Miotto P, Cabibbe AM, Borroni E, Degano M, Cirillo DM. Role of borderline rpoB mutations in rifampicin resistance. Clin Microbiol Infect. 2018;24(3):253–259.
- Vilhèze C, Jacobs WR Jr. Resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis*. Microbiol Spectr. 2014;2(4):MGM2-0014-2013.
- Hillemann D, Rüscher-Gerdes S, Richter E. Evaluation of GenoType MTBDRplus assay for detection of drug resistance. J Clin Microbiol. 2007;45(8):2635–2640.

- Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME. Rapid molecular screening for multidrug-resistant tuberculosis. *Diagn Microbiol Infect Dis*. 2008;60(4):337–343.
- Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampicin resistance. *N Engl J Med*. 2010;363(11):1005–1015.
- Dorman SE, Schumacher SG, Alland D, et al. Xpert MTB/RIF Ultra for detection of TB and rifampicin resistance. *Lancet Infect Dis*. 2018;18(1):76–84.
- Mokrousov I, Narvskaya O, Limeschenko E, et al. Real-time PCR assay for detection of katG mutations. *J Microbiol Methods*. 2002;51(2):283–291.
- Luo T, Yang C, Pang Y, et al. Development of multiplex real-time PCR for TB drug resistance detection. *Clin Microbiol Infect*. 2015;21(7):673.e1–673.e7.
- Pholwat S, Ehdaie B, Foongladda S, Houpt ER. Detection of heteroresistance using competitive wild-type blocking PCR. *J Clin Microbiol*. 2013;51(12):4014–4022.
- Avalos E, Catanzaro D, Catanzaro A, et al. Frequency and geographic distribution of gyrA mutations in fluoroquinolone-resistant TB. *Antimicrob Agents Chemother*. 2015;59(5):2851–2857.
- Maruri F, Sterling TR, Kaiga AW, et al. Systematic review of fluoroquinolone resistance mechanisms. *Emerg Infect Dis*. 2012;18(6):865–873.
- Farhat MR, Jacobson KR, Franke MF, et al. Genetic determinants of aminoglycoside resistance in TB. *Antimicrob Agents Chemother*. 2016;60(7):4145–4153.
- Tagliani E, Cabibbe AM, Miotto P, et al. Performance of GenoType MTBDRsl v2.0 assay. *Eur Respir J*. 2015;45(2):514–517.
- Penn-Nicholson A, Georghiou SB, Ciobanu N, et al. Diagnostic accuracy of Xpert MTB/XDR assay. *Lancet Infect Dis*. 2022;22(2):242–249.
- World Health Organization. Rapid communication: molecular assays for detection of TB drug resistance. Geneva: WHO; 2023.
- Andries K, Verhasselt P, Guillemont J, et al. Diarylquinoline drug bedaquiline targets ATP synthase. *Science*. 2005;307(5707):223–227.
- Nguyen TVA, Anthony RM, Bañuls AL, et al. Bedaquiline resistance mechanisms review. *Clin Microbiol Rev*. 2018;31(2):e00045-17.
- Ismail NA, Omar SV, Joseph L, et al. Bedaquiline resistance emergence in South Africa. *Lancet Microbe*. 2020;1(7):e270–e279.
- Doyle RM, Burgess C, Williams R, et al. Direct whole-genome sequencing for TB drug resistance prediction. *Clin Infect Dis*. 2018;66(12):1925–1932.
- Kohl TA, Utpatel C, Niemann S. Whole-genome sequencing in TB diagnostics. *Clin Microbiol Infect*. 2018;24(6):593–599.