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NAVIGATING TUBERCULOSIS TREATMENT CHALLENGES OF GENETIC PERSPECTIVES AND DNA-BASED DETECTION: A LITERATURE REVIEW

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ABSTRACT

Tuberculosis remains a major global health problem, particularly because of the increasing burden of drug-resistant tuberculosis (DR-TB), including MDR-TB and XDR-TB. Resistance in Mycobacterium tuberculosis is driven mainly by spontaneous genetic mutations, adaptive evolution, tolerance mechanisms, and host microenvironmental factors, which complicate both treatment and diagnosis. This review aimed to examine recent advances in the genetic mechanisms underlying antibiotic resistance in M. tuberculosis and to evaluate the progress of DNA-based detection technologies for tuberculosis diagnosis and management. This literature review analyzed 49 scientific articles published between 2012 and 2026 to examine the molecular and genetic basis of drug resistance in M. tuberculosis. The study focused on identifying target gene mutations, efflux pump mechanisms, compensatory evolution, and heteroresistance. Furthermore, it evaluated the clinical application of advanced DNA-based diagnostic tools, including GeneXpert, line probe assays, targeted next-generation sequencing (tNGS), whole-genome sequencing (WGS), droplet digital PCR, and CRISPR-based detection. Information was qualitatively synthesized to compare the efficacy and limitations of these modern diagnostic strategies in global tuberculosis management. Drug resistance was primarily associated with de novo mutations in genes such as rpoB, katG, inhA, pncA, gyrA/gyrB, and rrs/eis, as well as compensatory mutations that preserve bacterial fitness. In addition, non-genetic tolerance, dormancy, and efflux pump overexpression contributed to bacterial survival. DNA-based technologies enabled faster and more comprehensive resistance detection than conventional methods, although limitations remained in sensitivity, mutation coverage, cost, and infrastructure requirements. Genetic mutations, evolutionary adaptation, and tolerance mechanisms form the core basis of drug resistance in M. tuberculosis. Integrating DNA-based diagnostics into clinical practice is essential to improve early resistance detection, support personalised treatment, and reduce the spread of drug-resistant tuberculosis.

Keywords: drug resistance; DNA detection; efflux pump; genetic mutation; mycobacterium tuberculosis; whole-genome sequencing

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INTRODUCTION

Tuberculosis (TB) remains one of the most significant infectious diseases worldwide, causing substantial morbidity and mortality despite the availability of effective treatment regimens(Nimmo, C et al., 2022). The disease is caused by Mycobacterium tuberculosis, a pathogen that continues to pose a major challenge to global public health due to its ability to persist, spread, and develop resistance to antitubercular drugs (Anes, E., et al., 2023). The growing emergence of drug-resistant tuberculosis (DR-TB), including multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), has made TB control increasingly difficult (Pal, R., Bisht, M. K., & Mukhopadhyay, S., 2022). Resistance to first-line and second-line drugs limits treatment options, prolongs therapy, increases costs, and worsens patient outcomes. In many settings, delayed diagnosis and inappropriate empirical treatment further contribute to ongoing transmission and the expansion of resistant strains (Borah, P. et al., 2021).

At the molecular level, resistance in M. tuberculosis is driven mainly by spontaneous genetic mutations rather than horizontal gene transfer. Mutations in genes such as rpoB, katG, inhA, pncA, gyrA, and gyrB alter drug targets or activation pathways, reducing the effectiveness of major antitubercular agents (Pshennikova, E. S., & Voronina, A. S., 2022). In addition, non-genetic mechanisms such as efflux pump overexpression, dormancy, and phenotypic tolerance also support bacterial survival under antibiotic pressure (Canalda-Baltrons, A et al., 2025). Recent advances in DNA-based diagnostic technologies have improved the ability to detect TB and identify resistance more rapidly and accurately. (MacGregor-Fairlie et al., 2020) Methods such as GeneXpert, line probe assays, targeted next-generation sequencing, whole-genome sequencing, droplet digital PCR, and CRISPR-based diagnostics are transforming TB diagnosis and surveillance. These tools offer important opportunities for earlier detection, more precise treatment selection, and better control of resistant TB (Chauhan, M et al., 2024).

This review discusses the genetic basis of drug resistance in M. tuberculosis and highlights the role of modern DNA-based detection methods in improving TB management. By understanding these molecular mechanisms and diagnostic advances, more effective strategies can be developed to address the ongoing challenge of drug-resistant tuberculosis (Datta, D et al., 2024). This review discusses the genetic basis of drug resistance in M. tuberculosis and highlights the role of modern DNA-based detection methods in improving TB management. The objective of this study is to examine the recent advances in genetic mechanisms underlying antibiotic resistance specifically mutations in target genes and compensatory evolution and to evaluate the progress of DNA-based detection technologies, such as NGS and CRISPR, in enhancing diagnostic accuracy and personalized treatment for drug-resistant tuberculosis. By understanding these molecular mechanisms and diagnostic advances, more effective strategies can be developed to address the ongoing challenge of drug-resistant tuberculosis.

METHOD

This study employed a literature review design to examine the genetic basis of antibiotic resistance in Mycobacterium tuberculosis and the evolution of contemporary DNA-based diagnostic approaches. Data collection involved a systematic search of reputable scientific databases, including PubMed, Scopus, and Google Scholar. The selected literature comprised empirical research and review articles published between 2012 and 2026, ensuring the integration of the most recent advancements in genomic technology. (de Araujo, L et al., 2023). From an initial identification of 65 relevant publications, 49 articles were selected as primary sources based on their thematic alignment and methodological rigor. The review focused specifically on mutations within resistance-associated target genes such as rpoB, katG, inhA, pncA, gyrA, gyrB, rrs, and eis as well as broader mechanisms including efflux pump activity, compensatory evolution, dormancy, and heteroresistance. Additionally, the analysis evaluated the clinical application of advanced molecular diagnostic tools, including GeneXpert, line probe assays, targeted next-generation sequencing

(tNGS), whole-genome sequencing (WGS), droplet digital PCR, and CRISPR-based detection methods. (Dheda, K et al., 2024). All gathered information was qualitatively synthesized using a thematic approach to compare the strengths, limitations, and clinical relevance of each resistance mechanism and diagnostic efficacy. The findings were organized systematically to provide a comprehensive overview of early detection strategies and the future management of drug-resistant tuberculosis. (Dousa, K. M et al., 2020).

RESULT

Biological and Genetic Foundations of M. tuberculosis

The biological resilience of M. tuberculosis relies heavily on its unique and highly complex cell envelope, which creates a formidable barrier against both the host immune system and antibiotic penetration. This envelope features a core composed of a mycolic acid-peptidoglycan-arabinogalactan (mAGP) complex.11 The long, highly hydrophobic mycolic acid chains (C60-C90) are particularly critical for the bacterium's virulence and persistence, forming a thick, waxy protective layer that drastically limits cellular permeability. Above this core lies a peripheral lipid layer containing vital components like phthiocerol dimycocerosates (PDIM) and trehalose dimycolate (TDM), topped by an outer capsule of alpha-glucan and arabinomannan. This structural dynamic naturally confers a high degree of intrinsic resistance to the pathogen, explaining the necessity for long-term and complex therapeutic regimens to successfully eradicate the bacteria.

Beyond its physical defenses, M. tuberculosis utilizes a sophisticated array of virulence factors and proteins to manipulate the host's cellular environment, particularly within macrophages. A primary weapon is the ESX-1 Type VII secretion system, which facilitates the release of effector proteins such as the Early Secretory Antigenic Target 6 (ESAT-6) and Culture Filtrate Protein 10 (CFP-10).14,15 ESAT-6 functions as a porin that can lyse the phagosomal membrane, allowing the bacteria to escape degradation in the phagolysosome and enter the nutrient-rich cytoplasm of the host cell. Meanwhile, CFP-10 stabilizes ESAT-6 to ensure efficient secretion while also modulating host immune responses. Additionally, the bacterium dedicates nearly ten percent of its coding capacity to the PE (Proline-Glutamic acid) and PPE (Proline-Proline-Glutamic acid) protein families. These proteins are localized on the cell surface or secreted to subvert host immunity and dictate the fate of the infected macrophage, pushing it toward either protective apoptosis or tissue-damaging necroptosis depending on the stage of infection.

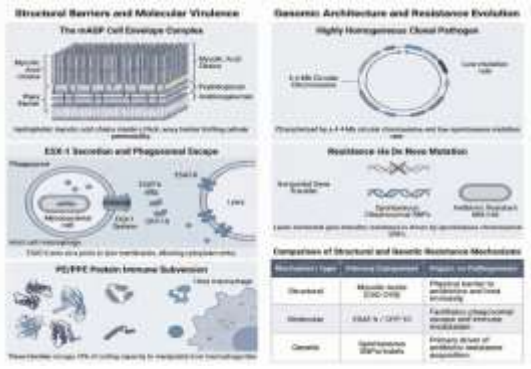

Figure 1. Mycobacterium Tuberculosis Pathogenesis and Resilience (Gengenbacher, M., & Kaufmann, S. H., 2012).

Molecular Mechanisms of Antibiotic Resistance

The molecular mechanisms underlying antibiotic resistance in M. tuberculosis are exceptionally complex and multifactorial, driven primarily by specific spontaneous genetic mutations that alter drug targets or interfere with drug activation pathways, as well as physiological adaptations to the host environment. Unlike many bacteria that rely on horizontal gene transfer, M. tuberculosis acquires hereditary resistance primarily through de novo chromosomal mutations such as single nucleotide polymorphisms (SNPs), insertions, and deletions which become fixed in the population

under the selective pressure of antibiotic therapy. The clinical consequences of these mutations depend heavily on the specific gene involved, the resulting increase in the minimum inhibitory concentration (MIC), and the overarching genomic context of the bacterial strain (Gygli, S. M., et al 2017).

Table 1.
Molecular Mechanisms and Diagnostic Profiles of Antibiotic Resistance in TB

Antibiotic Class	Target Gene/ Locus	Resistance Mechanism	Diagnostic Methodology	Clinical Classification	Fitness Cost & Compensatory Evolution (Inferred)
Rifamycins	rpoB	Target modification; RNA polymerase beta-subunit prevents drug binding	WGS, targeted sequencing (Deeplex-MycTB), Xpert MTB/RIF, LPA	MDR-TB, XDR-TB	Low fitness cost for S450L; compensatory mutations in rpoA or rpoC often restore fitness.
Isonicotinic acids	katG	Loss of pro-drug activation; prevents activation by catalase-peroxidase enzyme	WGS, Deeplex-MycTB, Xpert MTB/XDR, LPA	MDR-TB, XDR-TB, HR-TB	Minimal fitness cost; allows enzyme to maintain essential defensive functions against host ROS.
Diarylquinolines/Phenazines	mmpR5 (Rv0678)	Efflux pump overexpression; loss of repressor function leads to MmpL5/MmpS5 pump activity	WGS, targeted sequencing (Deeplex-MycTB)	XDR-TB	Moderate fitness cost; cross-resistance between Bedaquiline and Clofazimine often observed.
Isonicotinic acids	inhA (promoter)	Target modification/overexpression; reduces drug affinity and increases enzyme concentration	WGS, Deeplex-MycTB, Xpert MTB/XDR, LPA	MDR-TB, HR-TB	Generally lower fitness cost compared to katG deletions, leading to higher transmissibility.
Fluoroquinolones	gyrA / gyrB	Target modification; prevents drug binding to DNA gyrase	WGS, Deeplex-MycTB, Xpert MTB/XDR, LPA	Pre-XDR, XDR-TB	Some mutations in gyrA carry low fitness costs; compensatory mutations can occur in gyrB.
Aminoglycosides	rrs, eis	Target modification (rrs) or enzymatic inactivation (eis acetyltransferase)	WGS, LPA, Xpert MTB/XDR	XDR-TB	rrs mutations can significantly reduce translation efficiency; eis mutations often allow higher fitness.
Pyrazinamide	pncA	Loss of pro-drug activation; loss of pyrazinamidase (PZase) enzyme function	WGS, Deeplex-MycTB	MDR-TB	High variability in fitness cost depending on the specific mutation in the pncA gene.
Nitroimidazoles	ddn	Loss of pro-drug activation; results in non-functional proteins for Delamanid activation	WGS	MDR/XDR-TB	Variable; resistance emerges under selective pressure from prior drug exposure.

Evolutionary Adaptation and Genetic Diversity

Unlike many other bacterial species that rapidly acquire new survival traits through horizontal gene transfer, Mycobacterium tuberculosis rarely utilizes this mechanism to adapt to its environment.26 Integrating foreign genetic material carries a high risk of disrupting vital enzymatic functions, and such material is generally eliminated via purifying selection. Consequently, the evolutionary adaptation and acquisition of antibiotic resistance in M. tuberculosis rely almost entirely on the accumulation of spontaneous de novo chromosomal mutations that provide a selective advantage under the heavy pharmacological pressure of antibiotic therapy. While mutations conferring resistance typically impose a biological “fitness cost” that reduces the bacterium’s overall viability and replication speed, M. tuberculosis has evolved to elegantly counteract this burden through compensatory mutations. For example, the highly prevalent rpoB S450L mutation, which causes resistance to rifampicin, imposes minimal fitness loss when it is accompanied by secondary

compensatory mutations in the rpoA or rpoC genes. These secondary mutations stabilize the RNA polymerase complex and fully restore the bacterium's cellular fitness, allowing multidrug-resistant strains to survive, maintain high transmissibility, and spread efficiently across human populations without a biological disadvantage.

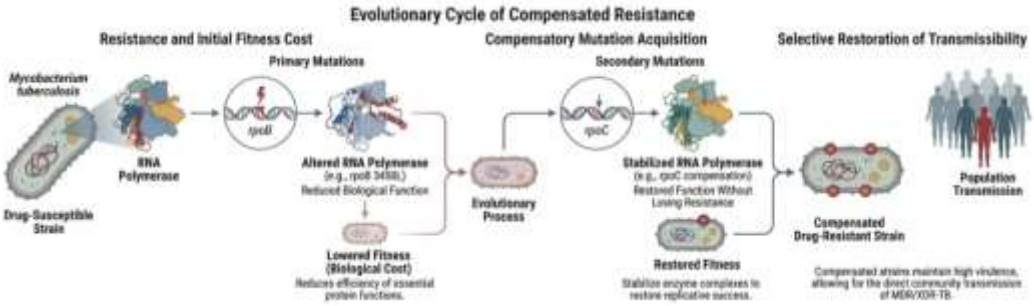

Figure 2. Evolutionary Dynamics of Fitness Compensation in Drug-Resistant Mycobacterium tuberculosis (Jose Vadakunnel, M et al., 2025)

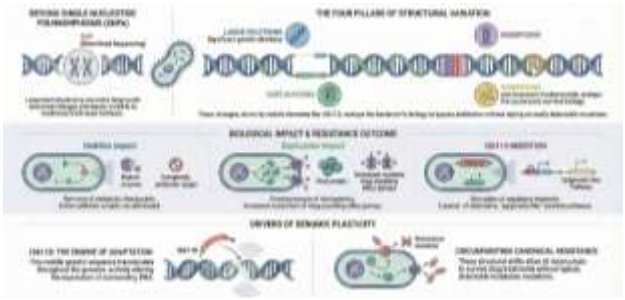

Figure 3. The Impact of IS6110-Driven Genomic Rearrangements on Survival Pathways and Resistance in Mycobacterium tuberculosis (Katoch, V. M., 2020)

Advancements in DNA-Based Detection Technologies

Table 2.
Comparison of current & emerging molecular diagnostic technologies for Mycobacterium tuberculosis

Kategori	Nama Teknologi	Keunggulan Utama	Keterbatasan Utama	Target Penanda
Cepat & Praktis (Point-of-Care)	Xpert MTB/RIF & XDR	Sangat cepat (<2 jam); mendeteksi bakteri sekaligus resistensi obat utama.	Biaya *cartridge* tinggi; hanya mendeteksi mutasi yang sudah diketahui.	*rpoB, katG, inhA, gyrA/B, rrs, eis*
	CRISPR-Cas12a	Sangat sensitif; hasil visual tanpa alat rumit; waktu <1,5 jam.	Masih tahap riset; risiko efek *off-target*.	IS6110
Sekuensing (Komprehensif)	Targeted NGS (tNGS)	Profil resistensi lengkap untuk 15 jenis obat; identifikasi ratusan spesies.	Tidak dapat mendeteksi mutasi baru di luar panel target.	15 Gen Resistensi
	Whole Genome Sequencing	Standar emas; memetakan seluruh genom bakteri secara utuh.	Waktu lama (~72 jam); biaya tinggi; butuh analisis bioinformatika rumit.	Seluruh Genom
	Oxford Nanopore	Alat portabel; mampu membaca struktur varian DNA yang kompleks.	Angka kesalahan pembacaan lebih tinggi dibanding Illumina.	Seluruh Genom
Lainnya	Line Probe Assay (LPA)	Efektif untuk skrining lini pertama & kedua secara manual.	Padat karya; interpretasi hasil bersifat subjektif.	*rpoB, katG, inhA, gyrA/B, rrs, eis*

Genotype-Phenotype Discordance and Heteroresistance

Table 3.
Genotype-Phenotype Discordance and Heteroresistance

Aspect	Findings	Clinical Implication
Genotype-phenotype discordance	DNA-based tests may detect resistance-associated mutations that do not always produce clear phenotypic resistance. Some isolates may appear susceptible in culture-based testing despite being genetically resistant	This may lead to underestimation of resistance and inappropriate treatment selection.
Low-level resistance	Certain mutations produce low-level resistance that may fall below the detection threshold of conventional phenotypic assays.	Patients may receive ineffective standard therapy, increasing the risk of treatment failure pmc.ncbi.nlm.nih.
Heteroresistance	Resistant and susceptible subpopulations can coexist in the same patient, with resistant fractions reported as low as 1% to 10% in some studies sciencedirect.	Minority resistant variants may be missed by routine testing, especially when bacterial load is low sciencedirect.
Conventional methods	Phenotypic drug susceptibility testing may fail to identify low-frequency resistant subpopulations.	Resistance may be recognised late, after resistance has expanded under treatment pressure
Molecular methods	Targeted NGS and ddPCR can detect mixed populations and low-frequency mutations more sensitively than conventional assays.	These methods improve early detection of emerging resistance and support more precise treatment decisions.
Overall impact	Discordance and heteroresistance complicate interpretation of TB drug-resistance results.	Integrated molecular and phenotypic assessment is needed for accurate diagnosis and regimen selection.

Clinical Challenges and Future Directions

Table 4.
Clinical Challenges and Future Directions

Aspect	Description	Implication
Resistance to newer drugs	Resistance is also emerging against bedaquiline, clofazimine, delamanid, and pretomanid.	Treatment options become more limited and complex.
Diagnostic difficulty in paucibacillary TB	Extrapulmonary TB, paediatric TB, and TB in HIV-coinfected patients often have very low bacterial loads.	Conventional tests may miss the infection or its resistance pattern.
Delay in appropriate therapy	Slow or incomplete diagnosis can lead to empirical treatment.	This increases the risk of treatment failure and ongoing transmission.
Need for molecular integration	Rapid molecular triage and sequencing-based profiling should be integrated into routine care.	Faster identification of resistance supports more accurate treatment selection.
Tiered diagnostic strategy	Use rapid point-of-care tests first, followed by advanced sequencing in reference laboratories.	This approach is practical and cost-effective, especially in low- and middle-income countries.
Drug optimisation	Molecular results can help avoid unnecessary use of toxic second-line drugs.	Reduces adverse effects and improves resource allocation.
Future drug discovery	Genetic engineering and functional genomics can validate essential bacterial targets.	Accelerates development of new antitubercular drugs.
Genomic surveillance	Ongoing monitoring of resistant strains and new mutations is needed.	Supports personalised treatment and public health control.

DISCUSSION

Biological and Genetic Foundations of M. tuberculosis

Mycobacterium tuberculosis has a thick, lipid-rich cell wall that makes it highly resistant to host immune responses and the penetration of antitubercular drugs. Genetically, the bacterium survives and adapts mainly through spontaneous mutations and the expression of virulence factors that support infection, survival, and persistence within the human body.

Molecular Mechanisms of Antibiotic Resistance

Resistance to primary first-line drugs, specifically rifampicin and isoniazid, constitutes the

fundamental barrier to successful tuberculosis treatment and defines the multidrug-resistant (MDR-TB) phenotype. Rifampicin functions by binding to the RNA polymerase beta-subunit to arrest mRNA synthesis, a process disrupted almost exclusively by mutations within the rpoB gene's 81-base pair region, predominantly the S450L substitution. Conversely, isoniazid resistance involves a more intricate genetic profile; as a prodrug, it requires activation by the KatG enzyme. The dominant katG S315T mutation bypasses this activation while uniquely preserving the enzyme's ability to defend the pathogen against host-generated oxidative stress, ensuring bacterial survival without compromising its physiological fitness. The therapeutic landscape further complicates as resistance emerges against second-line agents and novel compounds, such as fluoroquinolones, bedaquiline, and clofazimine. Fluoroquinolone resistance, a hallmark of extensively drug-resistant tuberculosis (XDR-TB), arises from mutations in the gyrA and gyrB genes that prevent drug binding to DNA gyrase. Beyond these specific genetic modifications, M. tuberculosis employs non-specific biological defenses, including drug-inactivating enzymes and the overexpression of the MmpL5/MmpS5 efflux pump system. Crucially, through the DosR regulon, the bacteria can transition into a non-replicating dormant state within host granulomas. This physiological persistence renders drugs targeting active metabolism ineffective and serves as an evolutionary bridge, allowing sub-populations to survive long enough to acquire the permanent genetic mutations that dictate absolute resistance.

Evolutionary Adaptation and Genetic Diversity

The resistance of M. tuberculosis is not merely a byproduct of individual genetic mutations but a result of sophisticated evolutionary adaptation. The pathogen exhibits significant genetic diversity, allowing sub-populations to survive diverse environmental pressures within the host. Central to this adaptation is the role of "fitness-neutral" mutations, such as the rpoB S450L substitution, which grants high-level resistance without compromising the bacterium's reproductive success. This stability is often reinforced by compensatory mutations in the rpoA or rpoC genes, which mitigate the physiological burden of resistance and ensure the long-term survival of multidrug-resistant strains.

Furthermore, the transition between genetic resistance and non-genetic tolerance represents a crucial evolutionary strategy. Through regulatory networks like the DosR regulon, the bacteria can enter a state of metabolic dormancy. This physiological persistence allows the pathogen to withstand prolonged antibiotic exposure in a non-replicating state, effectively serving as an evolutionary bridge. By surviving initial pharmacological assaults through these adaptive mechanisms, M. tuberculosis gains the necessary window to acquire permanent, high-level genetic mutations, thereby driving the continuous evolution of drug resistance.

Advancements in DNA-Based Detection Technologies

The rapid evolution of DNA-based diagnostic tools has fundamentally shifted the paradigm of tuberculosis management from slow, culture-based methods to rapid molecular precision. Current advancements, particularly in Nucleic Acid Amplification Tests (NAATs) like Xpert MTB/RIF Ultra, allow for the simultaneous detection of M. tuberculosis and critical resistance markers within hours. The integration of Loop-mediated Isothermal Amplification (LAMP) further enhances diagnostic accessibility in resource-limited settings by eliminating the need for complex thermal cyclers, thereby bridging the gap between high-tech laboratory settings and point-of-care clinical practice. Furthermore, the emergence of Whole Genome Sequencing (WGS) and Targeted Next-Generation Sequencing (tNGS) offers an unprecedented depth of genetic insight. Unlike traditional assays that target specific loci, sequencing technologies provide a comprehensive profile of the bacterial genome, enabling the detection of rare or compensatory mutations that contribute to low-level resistance. This shift toward high-throughput genomic analysis not only improves individual treatment outcomes through personalized medicine but also enhances global surveillance of resistant strains. By identifying emerging resistance patterns in real-time, these DNA-based

technologies serve as a critical defense against the further escalation of XDR-TB and other highly resistant phenotypes.

Genotype-Phenotype Discordance and Heteroresistance

A significant challenge in modern tuberculosis diagnostics is the occurrence of genotype-phenotype discordance, where molecular results do not align with traditional drug susceptibility testing (DST). This discrepancy often arises from "silent" or synonymous mutations that molecular assays may flag as resistance markers despite the bacteria remaining phenotypically sensitive. Conversely, discordance occurs when clinical resistance is present, yet the specific genetic locus targeted by the assay remains wild-type. This suggests the involvement of alternative resistance mechanisms, such as mutations in unmapped regulatory regions or the hyperactivation of non-specific efflux pumps, which underscores the inherent limitations of targeted molecular diagnostics compared to the comprehensive nature of phenotypic DST. The complexity of resistance is further intensified by heteroresistance, a phenomenon characterized by the co-existence of drug-susceptible and drug-resistant M. tuberculosis subpopulations within the same patient. Heteroresistance represents an early evolutionary stage of resistance, often invisible to conventional diagnostic methods if the resistant fraction falls below the assay's detection threshold. This intra-host diversity poses a severe clinical risk; under the selective pressure of antibiotic treatment, the resistant subpopulation can rapidly expand, leading to treatment failure and the eventual stabilization of a fully resistant genotype. Addressing these diagnostic gaps requires the integration of high-sensitivity sequencing technologies capable of detecting low-frequency variants to ensure more accurate and personalized therapeutic interventions

Genotype-Phenotype Discordance and Heteroresistance

A significant challenge in modern tuberculosis diagnostics is genotype-phenotype discordance, where molecular results fail to align with traditional drug susceptibility testing (DST). This discrepancy manifests either as "false resistance" due to non-clinical synonymous mutations or, more critically, as "false sensitivity" where clinical resistance exists without mutations in targeted genetic regions. Such discordance suggests the involvement of alternative mechanisms, such as distal regulatory mutations or the hyperactivation of non-specific efflux pumps, which underscores the inherent limitations of current targeted molecular assays. The diagnostic landscape is further complicated by heteroresistance, the simultaneous presence of drug-susceptible and drug-resistant subpopulations within a single host. This intra-host diversity often remains undetected by standard DST if the resistant fraction is below the 1% threshold, leading to "stealth" resistance and treatment failure under antibiotic pressure. Addressing these gaps requires a shift toward high-sensitivity technologies like Targeted Next-Generation Sequencing (tNGS) and Deep Sequencing, which can detect low-frequency variants. Recognizing these nuances is a clinical imperative to prevent the amplification of resistance and improve outcomes for MDR-TB and XDR-TB patients.

Clinical Challenges and Future Directions

The clinical management of drug-resistant tuberculosis (DR-TB) faces persistent hurdles despite significant technological breakthroughs. One of the primary clinical challenges is the complexity of interpreting molecular data in the presence of rare or novel mutations. As diagnostic reliance shifts toward genetic detection, clinicians often encounter "variants of unknown significance" (VUS)—mutations whose actual impact on drug efficacy remains uncharacterized. This uncertainty can lead to suboptimal dosing or the premature abandonment of effective drugs. Furthermore, the global disparity in laboratory infrastructure remains a critical barrier; while high-income regions utilize comprehensive genomic sequencing, many high-burden countries still rely on basic smear microscopy or limited molecular assays, leading to delayed diagnoses and continued community transmission. Another pressing challenge is the biological reality of persister cells and intracellular sequestration. Even when genetic testing suggests susceptibility, the physiological state of M. tuberculosis within the protective environment of a granuloma can shield it from pharmacological

action. Current therapeutic regimens often fail to account for this metabolic dormancy, necessitating prolonged treatment durations that contribute to high default rates and the subsequent amplification of resistance.

CONCLUSION

Tuberculosis remains a profound global health crisis, heavily exacerbated by the rapid spread of multidrug-resistant and extensively drug-resistant strains. The remarkable resilience of Mycobacterium tuberculosis stems from its severe genetic plasticity, utilizing spontaneous chromosomal mutations, compensatory evolution to restore cellular fitness, and large-scale structural variants (such as gene deletions and duplications) to survive antibiotic pressure and evade traditional detection methods. To overcome these complex survival mechanisms, clinical management must shift toward advanced DNA-based diagnostic technologies. High-throughput platforms like Whole-Genome Sequencing (WGS), portable Nanopore devices, and ultra-sensitive CRISPR-Cas assays allow clinicians to rapidly map comprehensive resistance profiles and track localized transmission clusters in real-time. Simultaneously, cutting-edge genetic engineering tools such as the ORBIT recombineering system and multiplexed CRISPR interference (CRISPRi) are revolutionizing functional genomics by drastically accelerating the discovery and validation of novel therapeutic targets. By combining these advanced molecular innovations with emerging host-directed therapies that target host-pathogen protein interactions, and integrating them into modernized global health security frameworks, the international community can move decisively closer to the total eradication of tuberculosis.

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