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Beyond Genetic Resistance: Phenotypic Drug Tolerance, Persistence and Diagnostic Blind Spots in Mycobacterium Tuberculosis

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Abstract **Review Article**

Background: Despite major advances in molecular diagnostics, tuberculosis (TB) treatment failure and relapse continue to occur among patients classified as drug-susceptible. Current phenotypic and genotypic drug susceptibility testing (DST) frameworks are designed to detect heritable genetic resistance but fail to capture non-genetic bacterial survival strategies.

Objective: This review critically examines diagnostic blind spots arising from phenotypic drug tolerance and persistence in Mycobacterium tuberculosis and their implications for TB treatment, surveillance, and control.

Main Body: Drug tolerance and persistence are reversible phenotypic states that enable M. tuberculosis to survive bactericidal drug exposure without changes in minimum inhibitory concentrations (MICs). Tolerant population's exhibit reduced killing kinetics, while persisters represent dormant subpopulations capable of resuming growth after treatment cessation. These survival states evade standard phenotypic and genotypic diagnostics, contributing to genotype–phenotype discordance, prolonged therapy, relapse, and amplification of resistance. Underlying mechanisms include metabolic downregulation, activation of stress-response pathways, toxin–antitoxin systems, and host-induced environmental adaptation. The diagnostic and programmatic consequences of these blind spots are particularly pronounced in high-burden, resource-limited settings.

Conclusion: Beyond genetic resistance, phenotypic drug tolerance and persistence are critical determinants of TB treatment outcomes. Addressing these diagnostic blind spots requires a paradigm shift toward integrating phenotypic survival assessment into diagnostic, therapeutic, and surveillance frameworks to strengthen global TB control.

Keywords: Tuberculosis; Drug resistance; Drug tolerance; Persisters; Genotype–phenotype discordance; Diagnostics

INTRODUCTION

Tuberculosis (TB), caused by Mycobacterium tuberculosis, remains a leading cause of morbidity and mortality worldwide, with drug-resistant forms posing a significant public health threat (WHO, 2021). Phenotypic and genotypic drug susceptibility testing (DST) is central to treatment decision-making. Nevertheless, clinical evidence shows that treatment failure and relapse often occur in patients classified as drug-susceptible.

Emerging evidence points to phenotypic survival strategies, including drug tolerance and persistence, as critical contributors to poor treatment outcomes (Balaban et al., 2019; Lewis, 2010). These reversible states enable bacilli to survive bactericidal drug exposure without altering MICs, creating significant diagnostic blind spots.

Objective: This review aims to critically examine current TB diagnostic frameworks, highlight their limitations in detecting phenotypic drug tolerance and persistence, and discuss implications for therapy, surveillance, and public health programs.

LITERATURE REVIEW

Phenotypic Drug Susceptibility Testing

Phenotypic DST evaluates bacterial growth in the presence of anti-tubercular agents, providing direct evidence of resistance. Common platforms include solid culture and the MGIT system. While reliable for detecting established resistance, phenotypic DST is limited by long turnaround times, variability in critical concentrations, and inability to detect low-frequency resistant or phenotypically tolerant populations (WHO, 2021).

Genotypic Drug Susceptibility Testing

Molecular assays, including Xpert MTB/RIF, line probe assays, and whole-genome sequencing (WGS), identify mutations associated with drug resistance (Walker et al., 2015; CRyPTIC Consortium, 2018). WGS has emerged as a powerful tool for predicting drug resistance through identification of known and novel resistance-associated mutations. However, WGS fundamentally infers resistance based on genetic determinants and does not capture phenotypic drug tolerance or persistence, which are defined by altered killing dynamics rather than MIC changes. Consequently, WGS cannot reliably predict treatment failure driven by non-heritable survival states.

Genotype–Phenotype Discordance

Discordance between genetic and phenotypic resistance highlights a key diagnostic limitation. Resistance-associated mutations may not manifest phenotypically, whereas tolerant or persister populations survive therapy undetected (Balaban et al., 2019).

Heteroresistance and Mixed Infections

Heteroresistance, the coexistence of susceptible and resistant bacterial populations, complicates diagnosis. Standard assays may fail to detect minority resistant populations, resulting in misclassification and suboptimal therapy.

Drug Tolerance and Persistence

Drug tolerance refers to slowed bacterial killing without MIC change, whereas persisters are dormant subpopulations capable of resuming growth post-therapy. Mechanisms include metabolic downregulation, stress response activation, toxin–antitoxin systems, and altered cell wall permeability (Lewis, 2010; Balaban et al., 2019). TB-specific studies show rifampicin tolerance in intracellular bacteria exposed to macrophage stress, demonstrating clinical relevance (Adams et al., 2011; Aldridge et al., 2014).

From a diagnostic perspective, these survival strategies systematically evade detection, explaining many instances of treatment failure despite apparent susceptibility.

KEY FINDINGS AND ISSUES

Key Findings:

Current DST reliably detects genetic resistance but fails to identify reversible phenotypic survival states.

Drug tolerance and persistence significantly contribute to treatment failure and relapse.

Genotype–phenotype discordance reflects diagnostic blind spots rather than laboratory error.

Undetected phenotypic survivors may facilitate the emergence of genetic resistance.

Diagnostic gaps are amplified in high-burden, resource-limited settings.

Key Issues:

Binary DST classifications are insufficient to guide therapy.

Misclassification leads to prolonged therapy and relapse.

Programmatic strategies must address both biological and diagnostic limitations.

DISCUSSION:

The complex interplay between genetic resistance, drug tolerance, and persistence necessitates a paradigm shift in tuberculosis diagnostics and treatment strategies. Current DST frameworks rely on binary susceptibility classifications that were never designed to capture dynamic phenotypic survival responses to antimicrobial stress (Balaban et al., 2019; Lewis, 2010). As a result, tolerant and persister populations remain undetected, despite their significant contribution to prolonged therapy, relapse, and resistance amplification (Adams et al., 2011; Aldridge et al., 2014).

Drug tolerance slows bacterial killing without altering MICs, while persisters represent transient dormant subpopulations capable of resuming growth after treatment cessation (Lewis, 2010; Balaban et al., 2019). These states are particularly problematic in TB, where prolonged treatment durations and heterogeneous lesion microenvironments favor phenotypic adaptation (McCune et al., 1966; Adams et al., 2011). Importantly, tolerant and persister populations provide a reservoir from which genetically resistant mutants may eventually emerge, linking phenotypic survival directly to the evolution of resistance (Aldridge et al., 2014; Balaban et al., 2019).

From a diagnostic perspective, both phenotypic and molecular assays systematically miss these survival states. Culture-based DST measures growth inhibition at fixed time points, while molecular diagnostics—including WGS—identify only heritable resistance determinants (Walker et al., 2015; CRyPTIC Consortium, 2018). This disconnect explains many instances of genotype–phenotype discordance and apparent “unexplained” treatment failure in drug-susceptible TB (Balaban et al., 2019).

Programmatically, the consequences are substantial. In high-burden settings, undetected phenotypic survivors lead to repeated treatment cycles, extended infectiousness, increased healthcare costs, and sustained transmission (WHO, 2021). As TB control strategies increasingly rely on rapid molecular diagnostics and shorter regimens, failure to account for phenotypic survival risks undermining recent gains in TB care.

Future diagnostic frameworks should integrate assessments of bacterial killing dynamics, such as time–kill assays, minimum duration for killing (MDK), and host-mimicking phenotypic DST platforms. Computational modeling and systems biology approaches, combined with WGS, may further enable prediction of survival-prone phenotypes and guide precision TB therapy (Balaban et al., 2019; Aldridge et al., 2014).

CONCLUSION

Beyond genetic resistance, phenotypic drug tolerance and persistence are critical determinants of TB treatment outcomes. Standard diagnostics fail to capture these states, contributing to treatment failure, relapse, and eventual resistance emergence. Addressing these diagnostic blind spots requires a paradigm shift toward integrating phenotypic survival assessment into diagnostic, therapeutic, and surveillance frameworks, thereby improving treatment efficacy and strengthening global TB control.

RECOMMENDATIONS:

Develop and validate diagnostics capable of detecting tolerant and persister populations.

Optimize treatment regimens to target phenotypic survival states.

Incorporate phenotypic survival metrics into TB surveillance frameworks.

Leverage computational modeling and WGS for predictive analysis of survival states.

Prioritize interventions in high-burden, resource-limited settings.

Policy Translation for Nigeria- Integrate phenotypic survival assessment: Nigeria's TB programs could adopt time–kill assays or host-mimicking DST to detect tolerance/persistence.

- Target high-burden areas: Focus on states like Lagos, Kano, Kaduna with high TB burdens.
- Strengthen lab capacity: Enhance GeneXpert/MGIT use + train on phenotypic DST interpretation.
- Treatment optimization: Adjust regimens for patients with suspected tolerance/persistence.
- Surveillance: Include phenotypic survival metrics in Nigeria's TB surveillance frameworks.

Nigeria-Specific Recommendations

1. Collaborate with NCDC/NTB Program to pilot phenotypic DST in key states.

2. Train healthcare workers on interpreting tolerance/persistence data.

3. Link findings to Nigeria's National TB Strategic Plan (2021–2025).

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