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Integrated Phenotypic and Genotypic Drug Susceptibility Profiling of *Mycobacterium tuberculosis* Reveals High Burden of Multidrug Resistance in Northwest Nigeria

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Abstract

Tuberculosis (TB) drug resistance remains a major public health challenge in Northwest Nigeria, undermining effective disease control and treatment outcomes. This study aimed to assess the genotypic and phenotypic drug susceptibility patterns of *Mycobacterium tuberculosis* isolates from patients attending TB Reference Centres, with particular focus on first-line, second-line, repurposed, and new anti-TB drugs. A cross-sectional design was employed involving 503 presumptive TB cases. MTB detection and rifampicin resistance were screened using Xpert MTB/RIF Ultra, genotypic resistance was assessed using Line Probe Assay, and phenotypic susceptibility was evaluated through Lowenstein–Jensen culture and MGIT Bactec 960. Associations between conventional drugs, repurposed drugs (levofloxacin, moxifloxacin, and linezolid), and the new drug bedaquiline with multidrug-resistant TB were analyzed using chi-square tests. The results confirmed MTB in 122 patients (24.3%), including 34 rifampicin-resistant cases (28.0%). Genotypic testing

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showed that 11 isolates (9.0%) were resistant to at least one drug, whereas 111 isolates (90.9%) were pan-susceptible. The prevalence of multidrug-resistant TB was 17.9%, with high susceptibility to levofloxacin (95.9%) and moxifloxacin (97.5%). Chi-square analysis demonstrated statistically significant associations between conventional, repurposed, and new anti-TB drugs and multidrug-resistant TB ($p < .001$). Discordance between phenotypic and genotypic drug susceptibility testing was also observed. Geographically, TB hotspots were identified in Sokoto, Kaduna, and Kano, while HIV co-infection remained low (4.4%). These findings indicate that a substantial proportion of TB patients harbor drug-resistant *Mycobacterium tuberculosis* strains, highlighting the need for routine drug susceptibility testing, continuous monitoring of new and repurposed drug effectiveness, strengthened regional surveillance, and evidence-based TB control policies to improve treatment decisions and curb the spread of multidrug-resistant TB.

Keywords: Drug Susceptibility Testing; Line Probe Assay; Multidrug-Resistant Tuberculosis; *Mycobacterium tuberculosis*; Northwest Nigeria

Introduction

Tuberculosis (TB) remains one of the most devastating infectious diseases globally, with drug-resistant forms posing a serious threat to disease control and elimination efforts [1]. Despite advances in diagnostics and chemotherapy, the emergence and sustained transmission of drug-resistant *Mycobacterium tuberculosis*(MTB), particularly multidrug-resistant tuberculosis (MDR-TB) and rifampicin-resistant tuberculosis (RR-TB), continue to undermine treatment success, increase mortality, and place substantial pressure on health systems [2][3]. These challenges are most pronounced in high-burden, resource-limited countries such as Nigeria, where under-diagnosis, delayed detection of resistance, and limited access to comprehensive drug susceptibility testing (DST) contribute to ongoing transmission of resistant strains.

Nigeria is consistently listed by the World Health Organization (WHO) among countries with the highest burdens of TB, TB/HIV co-infection, and MDR/RR-TB [4]. Recent estimates suggest approximately 479,000 new TB cases and about 12,000 MDR/RR-TB cases annually, with under-reporting and under-diagnosis remaining major obstacles to effective control [5][6][7]. The Northwest geopolitical zone of Nigeria represents a critical hotspot due to its large population, high mobility, and uneven

laboratory coverage. Routine surveillance data indicate variability in TB detection and rifampicin resistance rates across states in this region, raising concerns about undetected resistance and inappropriate treatment, which may further drive the spread of MDR-TB [8].

In a preliminary investigation, we previously reported the prevalence of rifampicin resistance and multidrug-resistant tuberculosis (MDR-TB) among patients attending tuberculosis reference centres in Northwest Nigeria using conventional diagnostic approaches. However, that analysis did not integrate comprehensive genotypic–phenotypic drug susceptibility profiling or evaluate resistance to repurposed and newly introduced anti-tuberculosis drugs. The present study builds on these earlier findings by applying an integrated diagnostic framework to generate deeper mechanistic insights and policy-relevant evidence.

Rapid molecular diagnostics have become central to TB control strategies, with the WHO recommending nucleic acid amplification tests as the initial diagnostic approach [9][10][11]. In Nigeria, the National Tuberculosis, Leprosy and Buruli Ulcer Control Programme has expanded the use of GeneXpert MTB/RIF, with testing coverage increasing from 68% in 2022 to 71% in 2023 [12][13]. While this progress has improved the detection of MTB and rifampicin resistance, reliance on single molecular platforms remains insufficient. Molecular assays primarily detect known resistance-associated mutations and may fail to capture uncommon or emerging resistance mechanisms, while also providing limited information on susceptibility to second-line, repurposed, and newly introduced anti-TB drugs [14][15][16].

Phenotypic DST, based on culture methods such as Lowenstein–Jensen and the MGIT Bactec 960 system, remains the reference standard for assessing true drug susceptibility [17][18][19]. However, these methods are time-consuming and resource-intensive, often leading to diagnostic delays that can negatively affect patient outcomes. Increasing evidence of discordance between genotypic and phenotypic DST results particularly for rifampicin, fluoroquinolones, and newer drugs such as bedaquiline, highlights the limitations of relying on a single testing approach [20][21][22]. An integrated genotypic–phenotypic DST strategy is therefore essential to ensure accurate resistance detection, guide individualized treatment, and prevent the amplification of resistance.

The introduction of all-oral, bedaquiline-based regimens for drug-resistant TB in Nigeria has further increased the need for robust laboratory capacity to monitor resistance to both conventional and newer anti-TB drugs [23][24]. However, data on resistance patterns to second-line, repurposed, and new agents remain limited in Northwest Nigeria. Most routine diagnostics focus on rifampicin resistance as a surrogate marker for MDR-TB, leaving critical gaps in understanding resistance to fluoroquinolones, aminoglycosides, and newer therapeutic agents [25][26]. This lack of comprehensive resistance data limits evidence-based treatment selection and weakens programmatic planning and surveillance.

In this context, the present study was conducted to provide a comprehensive assessment of genotypic and phenotypic drug susceptibility patterns of MTB isolates from patients attending tuberculosis reference centres in Northwest Nigeria. By integrating molecular and culture-based DST approaches, this study aims to determine the prevalence and patterns of resistance to first-line, second-line, repurposed, and new anti-TB drugs, identify regional resistance hotspots, and evaluate the extent of discordance between testing methods. The findings are expected to generate evidence that will strengthen regional surveillance, support optimized treatment regimens, and inform national TB control policies aimed at reducing the burden and transmission of drug-resistant TB in Nigeria.

Materials and Methods

1. Study Design and Ethical Approval

This study was a national, cross-sectional laboratory-based investigation conducted between May and September 2024 to assess the genotypic and phenotypic drug susceptibility profiles of *Mycobacterium tuberculosis* (MTB) isolates in Northwest Nigeria. The study was implemented through the National Tuberculosis Reference Laboratory located at the National TB Training Centre, Saye, Zaria, Kaduna State, which serves as the national reference laboratory for TB diagnosis and drug resistance surveillance.

Ethical approval for the study was obtained from the National TB Reference Laboratory Research Ethics Committee in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patient data were anonymized, and laboratory analyses were performed using coded identifiers to ensure confidentiality.

2. Study Population and Definitions

The study population comprised newly registered and previously treated bacteriologically confirmed pulmonary TB patients attending Directly Observed Treatment Short-course (DOTS) centres across the Northwest geopolitical zone of Nigeria. Participants were enrolled based on the World Health Organization (WHO) 2013 revised definitions and reporting framework for tuberculosis [27]. New TB cases were defined as patients with bacteriologically confirmed TB who had never received anti-TB treatment or had taken anti-TB drugs for less than four weeks [28][29]. Previously treated TB cases included patients who had received one month or more of anti-TB treatment in the past and were further classified as relapse cases, treatment after failure, treatment after loss to follow-up, or other previously treated cases with undocumented outcomes [30]. A bacteriologically confirmed pulmonary TB case was defined as an individual with sputum positive for *M. tuberculosis* complex by smear microscopy, culture, or a WHO-approved rapid diagnostic test, including Xpert MTB/RIF [31][32]. All eligible patients from participating states had equal opportunity for inclusion based on disease burden and referral patterns.

3. Sample Collection and Processing

The sample size for this study was determined using a standard prevalence-based formula for large populations, as previously described by Pourhoseingholi *et al.*, (2013). A prevalence estimate of 50% for bacteriologically confirmed tuberculosis was applied, with a 95% confidence level and a precision of 5%. This yielded a minimum sample size of 453 specimens. To account for potential attrition, an additional 10% was included, resulting in a total sample size of 503. A total of 503 sputum samples were collected from eligible patients using simple random sampling across DOTS facilities in seven states of Northwest Nigeria [34]. Early morning sputum samples of good quality (purulent or mucoid) and a minimum volume of 2 mL were collected aseptically in sterile, wide-mouthed sputum containers. Samples were triple-packaged and transported within six hours of collection to the National TB Reference Laboratory under cold-chain conditions using glycerine ice-packed cooler boxes.

Upon receipt, sputum specimens were processed using standard N-acetyl-L-cysteine–sodium hydroxide (NALC–NaOH) decontamination and digestion procedures as recommended by the National Tuberculosis, Leprosy and Buruli Ulcer Control

Programme. Processed sediments were cultured on Lowenstein–Jensen media and incubated at 37 °C for up to eight weeks with weekly monitoring for growth. Acid-fast bacilli (AFB) smear microscopy using Ziehl–Neelsen staining was performed for initial confirmation [35].

Isolates exhibiting characteristic mycobacterial growth were further identified using a rapid immunochromatographic assay targeting the MPT64 antigen to differentiate *Mycobacterium tuberculosis* complex from non-tuberculous mycobacteria [36][37]. Confirmed MTB isolates were preserved for downstream genotypic and phenotypic drug susceptibility testing. Drug susceptibility testing included first-line, second-line, repurposed, and new anti-TB drugs, namely isoniazid, rifampicin, ethambutol, moxifloxacin, levofloxacin, kanamycin, amikacin, capreomycin, clofazimine, linezolid, and bedaquiline, using WHO- and CLSI-recommended protocols.

4. Phenotypic Drug Susceptibility Testing

Phenotypic drug susceptibility testing (DST) was performed on confirmed *Mycobacterium tuberculosis* complex isolates using both solid and liquid culture-based methods in accordance with National TB and WHO-recommended protocols [38]. Primary isolates were cultured on Lowenstein–Jensen (LJ) medium and incubated at 37 °C for up to eight weeks with weekly monitoring for growth [39][40]. Susceptibility to first-line anti-tuberculosis drugs such as isoniazid, rifampicin, and ethambutol, was assessed using the proportion method on LJ medium. Standardized bacterial suspensions were prepared and adjusted to McFarland standard No. 1, followed by serial dilutions and inoculation onto drug-containing and drug-free control media. Growth on drug-containing media was compared with growth controls to determine resistance at critical concentrations [41]. Second-line and repurposed anti-TB drugs, including fluoroquinolones (levofloxacin and moxifloxacin), aminoglycosides (amikacin and kanamycin), capreomycin, clofazimine, and linezolid, were similarly tested using culture-based DST methods [3][42]. Where applicable, liquid culture systems (MGIT 960) were used to enhance detection sensitivity and reduce time to results [43]. Drug susceptibility results were interpreted based on established critical proportion thresholds.

5. Genotypic Drug Susceptibility Testing

Genotypic DST was conducted to detect genetic mutations associated with resistance to first- and second-line anti-TB drugs [44]. Initial screening for *M. tuberculosis*

and rifampicin resistance was performed directly on sputum samples using the GeneXpert MTB/RIF Ultra assay following the manufacturer's instructions. For further characterization, DNA was extracted from decontaminated sputum samples or cultured isolates using the GenoLyse kit (Hain Lifescience, Germany). Extracted DNA served as the template for polymerase chain reaction (PCR) amplification [45][46]. Line Probe Assays (LPA) were then performed using the GenoType MTBDRplus and MTBDRsl assays to detect resistance-associated mutations in the rpoB, katG, inhA, gyrA, gyrB, and rrs genes [47]. Reverse hybridization and colorimetric detection were carried out according to the manufacturer's protocols. Resistance was defined by the absence of wild-type bands and/or the presence of specific mutation bands corresponding to rifampicin, isoniazid, fluoroquinolone, and injectable drug resistance [48][49]. Results were interpreted using standardized reference charts provided with the assays.

6. Statistical Analysis

Data were compiled and analyzed using Microsoft Excel and standard statistical tools. Descriptive statistics were used to summarize demographic, clinical, and laboratory characteristics of the study population. Prevalence estimates of drug resistance were calculated with corresponding 95% confidence intervals using the Clopper–Pearson exact method. Associations between drug resistance patterns and key variables such as HIV status, treatment history, conventional, repurposed, and new anti-TB drugs, were evaluated using chi-square tests. A p-value of <0.05 was considered statistically significant. Agreement between phenotypic and genotypic DST results for isoniazid and rifampicin was assessed using Cohen's kappa statistic, with strength of agreement interpreted according to standard benchmarks. Sensitivity and specificity of genotypic DST were also calculated using phenotypic DST as the reference standard.

Results

1. Baseline Characteristics

A total of 503 bacteriologically confirmed pulmonary tuberculosis patients were included in this study. The study population was predominantly male, with 327 males (65.0%) and 176 females (35.0%). The mean age of participants was 35.07 ± 13.58 years, indicating that most cases occurred among young and middle-aged adults. Regarding HIV status, 22 patients (4.37%) were co-infected with HIV, while the majority, 427 (84.89%),

tested negative. HIV status was unknown for 54 participants (10.74%). With respect to tuberculosis treatment history, most patients were newly diagnosed cases (369), whereas 107 patients were on follow-up treatment at the time of enrolment. Geographically, cases were unevenly distributed across the Northwest geopolitical zone. Sokoto State accounted for the highest proportion of cases (223; 44.33%), followed by Kaduna (119; 23.66%) and Kano (63; 12.52%). Lower case numbers were recorded in Kebbi (46; 9.15%), Zamfara (36; 7.16%), Katsina (9; 1.79%), and Jigawa (7; 1.39%). Overall, these findings summarize the demographic, clinical, and geographic characteristics of the study population and corresponding *Mycobacterium tuberculosis* isolates (Table 1).

Table 1. Characteristics of the study population and MTB isolates (N = 503).

Characteristics	Categories	Frequency (%)
Sex	Female Male	176 (35%) 327(65%)
Age(years), mean ± SD		35 .07 ± 13.577
HIV status	Positive Negative Unknown	22 (4.37%) 427 (84.89%) 54 (10.74%)
TB Treatment History	Diagnosis Follow-up	369 107
STATE	Jigawa Kaduna Kano Kebbi Katsina Sokoto Zamfara	7 (1.39%) 119 (23.66%) 63 (12.52%) 46 (9.15%) 9 (1.79%) 223 (44.33%) 36 (7.16%)

2. MTB Detection and Demographics

MTB detection showed notable variation across key demographic and clinical characteristics of the study population. A higher proportion of MTB-positive cases was observed among males compared with females, reflecting the overall male predominance in the cohort. This pattern is consistent with established gender-related differences in tuberculosis exposure, health-seeking behavior, and occupational risk. With respect to HIV status, MTB detection was identified among both HIV-positive and HIV-negative individuals, with the majority of cases occurring in patients who tested negative for HIV. However, MTB was also detected among TB/HIV co-infected patients, underscoring the continued burden of tuberculosis in immunocompromised populations and the need for

integrated TB–HIV screening and management strategies. Analysis based on treatment history revealed that MTB detection was more frequent among newly diagnosed patients than among those on follow-up treatment. Nevertheless, a substantial proportion of MTB-positive cases was identified among previously treated individuals, highlighting the persistence of active infection in this group and emphasizing the importance of routine monitoring and drug susceptibility testing during follow-up care.

3. Phenotypic Drug Resistance Patterns

Phenotypic drug susceptibility testing revealed varying levels of resistance among *Mycobacterium tuberculosis* (MTB) isolates to both first- and second-line anti-tuberculosis drugs. Among the 503 isolates assessed, 16.1% (81/503) demonstrated resistance to at least one first-line drug, while the majority, 83.9% (422/503), were fully susceptible to all tested drugs (Table 2). Rifampicin showed the highest prevalence of mono-resistance at 5.2% (26/503), followed by isoniazid at 3.0% (15/503) and ethambutol at 0.2% (1/503). Combined resistance to two first-line drugs was mainly limited to isoniazid and rifampicin, detected in 7.2% (36/503) of isolates, while triple resistance to isoniazid, rifampicin, and ethambutol occurred in 0.6% (3/503). No isolates exhibited resistance to all first-line drugs or to other dual-drug combinations.

Table 2. First-Line Phenotypic drug susceptibility patterns of MTB isolates.

Tested Drugs	Frequency N (%)
Any Resistance to one drug	
Any EMB	4 (0.8)
Any INH	54 (10.8)
Any RIF	65 (13.0)
Resistance to only one drug	
EMB Only	1 (0.2)
INH Only	15 (3)
RIF Only	26 (5.2)
Resistance to only two drugs	
INH +RIF	36 (7.2)
Resistance to only three drugs	
INH + RIF + EMB	3 (0.6)
No Resistance	422 (83.9)

Resistance to second-line anti-tuberculosis drugs was comparatively low. Of the 503 isolates tested, only 1.8% (9/503) showed resistance to at least one second-line drug, with kanamycin and levofloxacin being the most affected (1.0% each). Amikacin and capreomycin resistance occurred in 0.4% of isolates, while ethionamide resistance was observed in 0.2%. Mono-resistance to individual second-line drugs was rare (0.2% each), and combined resistance patterns, such as kanamycin plus capreomycin (0.2%), kanamycin plus levofloxacin (0.4%), and levofloxacin plus moxifloxacin (0.2%), were infrequent. Triple-drug resistance was detected in 0.2% of isolates for both amikacin–levofloxacin–moxifloxacin and kanamycin–capreomycin–ethionamide combinations. No isolate exhibited resistance to all tested second-line drugs (Table 3).

Table 3. Second-Line Phenotypic drug susceptibility patterns of MTB isolates.

Tested Drugs	Frequency N (%)
Any Resistance to one drug	
Any AM	2 (0.4)
Any CM	2 (0.4)
Any ETO	1 (0.2)
Any KM	5 (1.0)
Any LFX	5 (1.0)
Resistance to only one drug	
AM Only	1 (0.2)
KM Only	1 (0.2)
LFX Only	1 (0.2)
Resistance to only two drugs	
KM + CM	1 (0.2)
KM + LFX	2 (0.4)
LFX +MFX	1 (0.2)
Resistance to only three drugs	
AM + LFX +MFX	1 (0.2)
KM + CM + ETO	1 (0.2)
No Resistance	494 (98.2)

Further analysis of 122 MTB isolates specifically assessed for both first- and second-line drug resistance showed that 26% (32/122) were classified as multidrug-resistant TB (MDR-TB), exhibiting simultaneous resistance to both isoniazid (INH) and

rifampicin (RIF). Additionally, 17% were RIF-resistant and 5% were INH-resistant as mono-resistant cases, while 52% of isolates were pan-susceptible to first-line drugs (Fig 1). For second-line drugs, 9% (11/122) met the criteria for pre-extensively drug-resistant TB (pre-XDR-TB), indicating resistance to fluoroquinolones in addition to MDR, and no cases of extensively drug-resistant TB (XDR-TB) were detected. The majority, 91% (111/122), were pan-susceptible to second-line drugs (Table 4).

Table 4. Occurrence (resistance) of Mycobacterium tuberculosis detected by the Phenotypic First and Second Line drug susceptibility (DST) test Method.

Mycobacterium tuberculosis detected by Phenotypic First Line DST	**Occurrence**	**Rate (%)**
MDR TB	32	26
RIF Resistance	21	17
INH Resistance	6	5
Pan Susceptible	63	52
Total	122	100
Mycobacterium tuberculosis detected by Phenotypic Second Line DST	Occurrence	Rate (%)
Pre-XDR TB	11	9
Pan Susceptible to Second Line	111	91
XDR TB	0	0
Total	122	100

Overall, these findings highlight the clinical challenge posed by MDR- and pre-XDR-TB in Northwest Nigeria while confirming that the majority of MTB strains remain susceptible to standard first-line therapy. This underscores the importance of routine phenotypic drug susceptibility testing, continuous surveillance, and timely detection of emerging drug resistance to guide effective treatment strategies.

4. Genotypic Resistance Patterns

Genotypic drug-resistance profiling using the Line Probe Assay (LPA) revealed the presence of resistance-associated mutations among a substantial proportion of *Mycobacterium tuberculosis* (MTB) isolates (Table 3.4). Of the 122 samples in which MTB DNA was detected, 22 isolates were identified as multidrug-resistant tuberculosis (MDR-TB), indicating concurrent resistance to rifampicin (RIF) and isoniazid (INH). Within this MDR-TB group, RIF resistance was more frequently detected than INH resistance, with 17 and 5 cases, respectively. The LPA further identified resistance to second-line anti-

tuberculosis drugs, with mutations associated with fluoroquinolone (FLQ) resistance detected in eight isolates and aminoglycoside (AMG) resistance detected in two isolates. These findings indicate that while first-line drug resistance predominates, resistance to second-line agents particularly FLQs, which are critical in MDR-TB management, is also present, albeit at lower frequencies. The assay additionally distinguishes wild-type strains, reflecting the absence of detectable resistance-associated mutations, thereby underscoring its utility in rapidly differentiating susceptible and resistant MTB strains.

Table 5. Genotypic Detection and Drug-Resistance Profile of Mycobacterium tuberculosis Using Line Probe Assay

Parameter / Classification	Number (n)	Notes
Total MTB detected	122	MTB DNA present
MDR-TB detected	22	Resistance to both RIF & INH
• RIF-resistant	17	Subset of MDR-TB
• INH-resistant	5	Subset of MDR-TB
Second-line resistance	—	—
• FLQ-resistant	8	Fluoroquinolone resistance
• AMG-resistant	2	Aminoglycoside resistance
Wild-type (No resistance mutations)	Not stated	No detectable mutations

Genotypic resistance patterns were further assessed using GeneXpert MTB/RIF and Xpert MTB-DRS assays (Table 6). Among the 503 presumptive TB samples tested, 122 (24.3%) were confirmed as MTB-positive by GeneXpert MTB/RIF. Of these MTB-positive cases, 34 isolates (27.9%) harbored mutations conferring rifampicin resistance. Extended genotypic testing with Xpert MTB-DRS demonstrated a high burden of resistance to isoniazid and second-line drugs, with resistance detected in 77% of cases subjected to DRS analysis. This high proportion highlights a considerable prevalence of drug-resistant MTB strains among the tested population.

Table 6. Occurrence and Drug-Resistance Pattern of Mycobacterium tuberculosis Detected by GeneXpert MTB/RIF and Xpert MTB-DRS

Parameter / Classification	Number (n)	Percentage (%)	Notes
Total samples tested	503	100	All presumptive TB samples
MTB-positive by Xpert MTB/RIF	122	24.3	MTB DNA detected
RIF-resistant MTB detected	34	27.9*	Among 122 MTB-positive
Resistance to INH & 2nd-line drugs (Xpert MTB-DRS)	—	77	Based on genotypic DST
MTB-susceptible (no resistance)	—	—	Not reported

*Percentage calculated: 34 / 122 × 100 ≈ 27.9%

Collectively, the GeneXpert-based findings corroborate the LPA results, emphasizing the substantial contribution of genotypic methods in the rapid detection of both first-line and second-line drug resistance and in informing early treatment decisions.

6. Comparison of Phenotypic versus Genotypic Drug Susceptibility Testing

The level of agreement between phenotypic and genotypic drug susceptibility testing (DST) methods for isoniazid and rifampicin is summarized in Table 7. Overall, the concordance between the two approaches ranged from fair to moderate. For isoniazid, the inter-rater agreement was fair, with a kappa value of 0.476 (95% CI: 0.39–0.56), while rifampicin showed a moderate but lower level of agreement, with a kappa value of 0.391 (95% CI: 0.30–0.48). Concordant susceptible–susceptible results accounted for 75.4% (66/96) of INH cases and 57.3% (51/89) of RIF cases, indicating reasonable alignment between the two methods in identifying susceptible isolates.

Table 7. Comparison between Phenotypic and Genotypic drug susceptibility testing

Type of drug		Genotypic Resistant	Genotypic Susceptible	Kappa Test
INH	Phenotypic Resistant	26	28	Kappa = 0.476 (95 % CI: 0.39 - 0.56)
	Phenotypic Susceptible	2	66	
RIF	Resistant	33	32	Kappa = 0.391 (95 % CI: 0.30 - 0.48)
	Susceptible	6	51	

Despite this overall agreement, notable discordance was observed. Resistant–susceptible discrepancies were recorded in 30.4% of INH cases and 38.1% of RIF cases, suggesting that genotypic methods detected resistance-associated mutations in isolates that were classified as susceptible by phenotypic DST. A smaller number of susceptible–resistant discrepancies were also observed for both drugs. Sensitivity and specificity analyses showed that genotypic DST demonstrated higher sensitivity for detecting INH resistance (92.9%) but lower specificity (70.2%), whereas for rifampicin, sensitivity and specificity were 84.6% and 61.4%, respectively. These findings indicate that while genotypic methods offer rapid and sensitive detection of resistance, discrepancies with phenotypic DST persist, particularly for rifampicin, highlighting the complementary roles of both approaches in comprehensive tuberculosis drug-resistance surveillance.

6. Repurposed and New Anti-TB Drugs

The resistance profile of repurposed anti-tuberculosis drugs among multidrug-resistant tuberculosis (MDR-TB) isolates is presented in Table 8. Analysis of fluoroquinolone susceptibility showed that resistance to levofloxacin and moxifloxacin was relatively uncommon among MDR-TB cases. Of the 32 confirmed MDR-TB isolates, only three (9.4%) were resistant to levofloxacin, while the remaining 29 were susceptible. Similarly, resistance to moxifloxacin was detected in just one MDR-TB isolate (3.1%), with the vast majority demonstrating susceptibility. Among non-MDR-TB cases, resistance to levofloxacin was detected in only two isolates, while no resistance to moxifloxacin was observed. These findings indicate a high level of retained activity of repurposed fluoroquinolones against MDR-TB strains in this setting, supporting their continued relevance in second-line treatment regimens. The low prevalence of fluoroquinolone resistance further suggests limited progression toward pre-extensively drug-resistant tuberculosis (pre-XDR-TB) within the study population.

Table 8. Prevalence of Repurposed anti-TB drugs among MDR Isolates

MDR	Levoflaxacin (R)	Levoflaxacin (S)	Moxiflaxacin (R)	Moxiflaxacin (S)	Total
No	**2**	**88**	**0**	**90**	**90**
Yes	3	29	1	31	32
Total	5	117	1	121	122

The association between resistance to conventional first-line anti-TB drugs and repurposed agents was evaluated using chi-square analysis. Highly significant associations were observed between rifampicin resistance and resistance to levofloxacin, moxifloxacin, clofazimine, and linezolid ($p < 0.001$ for all comparisons). Similar statistically significant associations were also identified between isoniazid resistance and each of the repurposed drugs examined. These results indicate that resistance to first-line drugs strongly correlates with resistance patterns observed for repurposed anti-TB agents. The large chi-square and likelihood ratio values, derived from a total sample size of 503 cases, demonstrate that these associations are robust and unlikely to be due to chance.

Table 9. Summary of Result of association between conventional and repurposed anti-TB drugs

Comparison	Chi-Square	df	Asymp. Sig. (2-sided)	Likelihood Ratio	N
Rifampicin * Levofloxacin	521.850	4	0.000	563.803	503
Rifampicin * Moxifloxacin	504.837	4	0.000	547.477	503
Rifampicin * Clofazimine	503.000	4	0.000	557.319	503
Rifampicin * Linezolid	503.000	4	0.000	557.319	503
Isoniazid * Levofloxacin	530.069	4	0.000	565.740	503
Isoniazid * Moxifloxacin	508.361	4	0.000	548.417	503
Isoniazid * Linezolid	503.000	4	0.000	557.319	503
Isoniazid * Clofazimine	503.000	4	0.000	557.319	503

Further analysis focusing specifically on confirmed MDR-TB status (Table 10) revealed a strong and highly significant relationship between MDR-TB and resistance to fluoroquinolones. Both levofloxacin and moxifloxacin showed p-values < 0.001, indicating that fluoroquinolone resistance is strongly associated with MDR-TB. Given the central role of fluoroquinolones in MDR-TB treatment, this finding is epidemiologically important, as it underscores the risk of MDR-TB strains evolving toward pre-XDR-TB or extensively drug-resistant TB (XDR-TB). The observed resistance may reflect prior fluoroquinolone exposure, inappropriate antibiotic use, or ongoing community transmission of resistant strains.

Table 10. Summary of Result of association between repurposed anti-TB drugs and confirmed MDR status.

Test	Levofloxacin	Moxifloxacin
Chi-Square	515.669	521.670
Likelihood Ratio	559.963	551.677
Df	4	4
Asymptotic Significance (2-sided)	0.000	0.000
N	503	503

The association between conventional first-line drugs and newer anti-TB agents was also examined (Tables 11 and 12). Resistance to rifampicin and isoniazid showed a highly significant association with resistance to bedaquiline, a key novel drug in MDR-TB management ($p < 0.001$). The very high chi-square and likelihood ratio values indicate a strong and consistent relationship between resistance to first-line agents and bedaquiline resistance. This finding suggests potential shared resistance pathways, treatment history effects, or selective pressure among patients with advanced drug-resistant TB.

Table 11. Summary of Result of association between new anti-TB drugs and confirmed MDR status

	Value	Df	Asymptomatic Significance(2-sided)
Chi-Square	503.000	2	.000
Likelihood Ratio	557.319	2	.000
N	503		

Table 12. Summary of Result of association between conventional and new anti-TB drug

Comparison	Chi-Square	Df	Asymp. Sig. (2-sided)	Likelihood Ratio	N
Rifampicin * Bedaquiline	503.000	2	0.000	557.319	503
Isoniazid * Bedaquiline	503.000	2	0.000	557.319	503

Collectively, these results highlight the importance of continuous surveillance of both repurposed and new anti-TB drugs to preserve their effectiveness and guide evidence-based MDR-TB treatment strategies.

Discussion

1. Key Findings

This study provides a comprehensive overview of the phenotypic and genotypic drug-resistance patterns of *Mycobacterium tuberculosis* (MTB) isolates from TB reference centres in Northwest Nigeria. The key findings include a moderate MTB detection rate (24.3%) among presumptive TB cases, a substantial burden of rifampicin resistance (27.9% among MTB-positive cases), and a notable prevalence of multidrug-resistant tuberculosis (MDR-TB). The MDR-TB prevalence observed in the present study is higher than that reported in our earlier preliminary analysis from the same region, likely reflecting the inclusion of expanded phenotypic and genotypic testing platforms as well as additional drug classes. Although the majority of isolates remained susceptible to first-line and second-line drugs, a concerning proportion of MDR-TB and pre-extensively drug-resistant TB (pre-XDR-TB) cases was identified. Genotypic assays revealed resistance-associated mutations to both first-line and second-line drugs, while phenotypic testing confirmed variable resistance patterns. Importantly, repurposed drugs such as levofloxacin and moxifloxacin, as well as the newer agent bedaquiline, largely retained activity, although their resistance profiles were significantly associated with MDR-TB status. Collectively, these findings spotlight the ongoing challenge of drug-resistant TB in the region and the need for sustained diagnostic and surveillance efforts.

2. Comparison with African and Global Data

The Mycobacterium tuberculosis (MTB) detection rate observed in this study is comparable to reports from other high-burden settings in sub-Saharan Africa, where positivity rates among presumptive TB cases typically range between 20% and 30%. For instance, Ibrahim *et al.* (2022) reported an MTB incidence of 22.68% among 2,451 presumptive TB cases in northeastern Nigeria, with rifampicin-resistant MTB detected in 4.5% of cases [53]. Similarly, Ulasi *et al.* (2022) found a rifampicin resistance prevalence of 6.8% among 1,300 presumptive multidrug-resistant TB patients in Enugu, Nigeria, highlighting ongoing transmission of resistant strains among young adults, particularly males [52]. Elsy Carvajal *et al.* (2025) further demonstrated that among 55 MTB isolates from Ibadan, Nigeria [54], mutations in rpoB, inhA, and gyrA genes were associated with phenotypic resistance to rifampicin, isoniazid, and fluoroquinolones, emphasizing the utility of molecular tools for local surveillance and early detection of resistance [50][51]. The

rifampicin resistance prevalence observed here aligns with these regional findings, illustrating heterogeneity across Nigerian states: in Enugu, rifampicin resistance was 6.8% (Ulasi *et al.*, 2022), in northeastern Nigeria 4.5% (Ibrahim *et al.*, 2022), and in Ibadan molecular analyses revealed notable rpoB-associated resistance (Carvajal *et al.*, 2025). Similarly, the MDR-TB prevalence documented in this study falls within the range reported in national surveys and WHO estimates for high-burden African countries

Ugwu *et al.* (2021) reported MDR-TB prevalence in Enugu at 2.8–4.6% depending on patient treatment history[4], while Tan *et al.* (2025) highlighted that globally, the age-standardized incidence rate of MDR-TB was 5.42 per 100,000 population, with rising trends in low- and middle-income regions[55]. These findings underscore the continuing public health challenge posed by MDR-TB in high-burden African settings.

The relatively low frequency of second-line drug resistance and absence of confirmed XDR-TB cases are encouraging and consistent with other African studies. Otchere *et al.* (2024) noted that West African TB programs are strengthening diagnostics, expanding access to quality care, and introducing novel drugs such as bedaquiline and delamanid, contributing to improved outcomes for drug-resistant TB [56]. Likewise, Ismail *et al.* (2019) reported low national prevalence of XDR-TB in South Africa (4.9%), with second-line drug resistance observed predominantly among retreatment cases, highlighting the effectiveness of improved surveillance and treatment strategies [57]. Although higher rates have been reported in parts of Eastern Europe and Asia, Seung *et al.* (2015) emphasized that MDR- and XDR-TB remain a significant challenge due to weak health systems and amplification of resistance through inadequate treatment[3], while Yapa *et al.* (2025) noted ongoing global disparities in DR-TB management and emphasized the need for enhanced diagnostics and person-centered interventions [58]. Compared with global data, Cegielski *et al.* (2015) demonstrated that early detection of fluoroquinolone and second-line injectable resistance significantly improved treatment outcomes[59], while Shi *et al.* (2020) showed that implementation of rapid molecular DST in China reduced time to culture conversion and increased treatment success from 47% to 68% [60]. Nasiri *et al.* (2025) reported that long-term treatment outcomes for MDR-TB have steadily improved over 15 years, with success rates of 68.4% for MDR-TB and up to 85.8% in selected cohorts using novel regimens [61]. The persistence of fluoroquinolone susceptibility in most MDR-TB isolates in this study suggests that treatment outcomes in this setting could be optimized if early detection and appropriate regimen selection are ensured.

3. Diagnostic Implications

The observed differences between phenotypic and genotypic drug susceptibility testing highlight important diagnostic implications [62][63]. While genotypic methods such as GeneXpert MTB/RIF and Line Probe Assay enabled rapid detection of MTB and resistance-associated mutations, moderate discordance with phenotypic DST was observed, particularly for rifampicin and isoniazid [64]. Genotypic DST demonstrated higher sensitivity but lower specificity, indicating that some mutations detected may not always translate into phenotypic resistance [65]. Conversely, phenotypic DST, though slower, remains essential for confirming functional drug resistance and detecting resistance mechanisms not covered by molecular assays [66][67]. These findings support the complementary use of both diagnostic approaches in routine TB management, especially in high-burden settings where rapid initiation of effective therapy must be balanced with accurate resistance profiling.

4. Threat of Pre-XDR and XDR-TB

The identification of pre-XDR-TB cases, characterized by MDR-TB with additional fluoroquinolone resistance, represents a significant public health concern [68][69]. Fluoroquinolones are cornerstone drugs in MDR-TB treatment, and resistance to these agents markedly reduces therapeutic options and increases the risk of treatment failure [70][71]. Although no XDR-TB cases were detected in this study, the strong association between fluoroquinolone resistance, MDR-TB, and resistance to newer agents such as bedaquiline raises concern about potential progression toward more extensive resistance patterns [72][73]. These findings highlight the need for strict antimicrobial stewardship, regulation of fluoroquinolone use, and continuous monitoring of resistance trends to prevent escalation from MDR-TB to pre-XDR or XDR-TB.

5. Strengths and Limitations

A major strength of this study is its large sample size and the integration of phenotypic and genotypic drug susceptibility testing, providing a robust and comprehensive assessment of MTB drug resistance. The inclusion of repurposed and new anti-TB drugs further enhances the clinical relevance of the findings. Additionally, the geographic coverage across multiple states in Northwest Nigeria offers valuable regional insights into TB resistance patterns. However, some limitations should be acknowledged. Not all MTB-positive samples underwent complete second-line and newer drug

susceptibility testing, which may have led to underestimation of resistance prevalence. The cross-sectional design also limits the ability to assess treatment outcomes or temporal trends in resistance. Furthermore, molecular assays were restricted to known resistance-associated mutations and may not have captured novel or uncommon resistance mechanisms. Despite these limitations, the study provides important evidence to inform TB control strategies and guide future research in Nigeria and similar high-burden settings.

Conclusion

This study demonstrates a substantial burden of drug-resistant *Mycobacterium tuberculosis* in Northwest Nigeria, with a notable prevalence of rifampicin resistance, multidrug-resistant tuberculosis (MDR-TB), and emerging pre-extensively drug-resistant TB (pre-XDR-TB). By integrating phenotypic and genotypic drug susceptibility testing, the study reveals that while the majority of MTB isolates remain susceptible to first-line and second-line drugs, a significant proportion harbor resistance that could compromise treatment outcomes if not detected early. The observed discordance between phenotypic and genotypic DST, particularly for rifampicin and isoniazid, highlights the limitations of relying on a single diagnostic approach and underscores the necessity of a complementary testing strategy.

From a policy perspective, these findings support the urgent integration of expanded molecular DST into routine TB care beyond rifampicin resistance alone. While GeneXpert MTB/RIF has substantially improved early detection of TB and rifampicin resistance, it is insufficient for comprehensive resistance profiling. Incorporating Line Probe Assays and targeted second-line molecular tests into routine diagnostic algorithms especially for high-risk and previously treated patients, would enable earlier identification of MDR-TB and pre-XDR-TB, facilitate individualized treatment, and reduce ongoing transmission. Strengthening laboratory capacity to deliver integrated phenotypic–genotypic DST should therefore be prioritized within national TB control programs. In addition, the demonstrated associations between resistance to conventional first-line drugs and resistance to repurposed and newer agents such as fluoroquinolones, linezolid, clofazimine, and bedaquiline emphasize the need for strengthened surveillance of these critical drugs. Although high susceptibility to repurposed fluoroquinolones and bedaquiline was observed, the strong linkage with MDR-TB signals a risk of resistance amplification if drug

use is not carefully monitored. National TB policies should therefore reinforce antimicrobial stewardship, regulate access to fluoroquinolones in the community, and ensure routine monitoring of resistance to repurposed and new anti-TB drugs to preserve their long-term effectiveness.

Future Directions

Future research should build on these findings by incorporating whole-genome sequencing (WGS) into TB drug-resistance surveillance in Nigeria. WGS would enable comprehensive characterization of resistance-associated mutations, detection of uncommon or novel resistance mechanisms, and improved understanding of transmission dynamics across the Northwest region [74][75]. Integrating WGS with routine molecular and phenotypic DST could provide a powerful platform for precision TB management and outbreak investigation. Longitudinal resistance tracking is also essential to monitor temporal trends in drug resistance and evaluate the impact of evolving treatment regimens, particularly all-oral, bedaquiline-based therapies. Prospective cohort studies following patients through treatment and post-treatment periods would help clarify the clinical significance of genotypic–phenotypic discordance, assess treatment outcomes, and identify predictors of resistance acquisition [76]. Such data are critical for informing adaptive TB control strategies, optimizing treatment guidelines, and ultimately reducing the burden of MDR-TB and preventing progression to pre-XDR and XDR-TB in Nigeria.

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Ethics statement

Ethical approval for this study was obtained from the National TB Reference Laboratory Research Ethics Committee, in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patient information was fully anonymized, and laboratory analyses were conducted using coded identifiers to ensure confidentiality and data protection.

Disclosure statement

No potential conflict of interest was reported by the author(s). An earlier preliminary report of related data was published in a local journal; the current study presents extended data, additional analyses, and updated interpretations.

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Data availability statement

All data will be made available upon reasonable request.

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