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RESEARCH ARTICLE

Geospatial Distribution and Genetic Drivers of Multidrug-Resistant Tuberculosis in the North West Province of South Africa: Disproportionate rpoB-Mediated Rifampicin Resistance and Its Treatment Implications

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Abstract:

Background:

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), remains a major public health challenge, especially in low- and middle-income countries. Primarily affecting the respiratory system, TB is transmitted via airborne droplets. First-line drugs, isoniazid (INH) and rifampicin (RIF), are crucial for treatment, but resistance—mediated by mutations in *rpoB, katG*, and *inhA*—leads to multidrug-resistant TB (MDR-TB), complicating disease control and treatment outcomes. **Aim of the study:** The aim of this study was to evaluate the distribution and MDR-TB patterns in the North West Province, with a focus on behavioural insights into rifampicin and isoniazid resistance and their treatment implications. **Methods**: Retrospective data were obtained from the NHLS Corporate Data Warehouse via the Academic Affairs and Research Management Systems. Data analysis was performed using Microsoft Excel and included descriptive statistics, Chi-square, Fisher's exacttest, and ANOVA. Results are presented in tables and figures. Ethical approval was granted by Health Science Research Ethics Committee (HSREC), with institutional permission from the NHLS Academic Affairs & Research Office. **Results:** A total of 340 *Mycobacterium tuberculosis* isolates were analysed.MDR-TB cases in the North West Province were unevenly distributed across catchment areas. Tshepong reported the highest burden (n = 155, 45.6%; MDR prevalence 61%), followed by Moses Kotane (n = 32, 13.2%; MDR 71.1%). Smaller facilities, including Rustenburg, Mafikeng, Gelukspan, and Nic Bodenstein, contributed fewer cases but often had high MDR prevalence (50–75%). Analysis of resistance-associated genes showed that *rpoB*, linked to rifampicin resistance, had the highest proportion of resistant isolates (n = 219, 64.4%), followed by *katG* for isoniazid resistance (n = 173, 50%). Chi-square analysis confirmed a significant difference in resistance distribution across *rpoB, katG,* and *inhA* ($\chi^2(2) = 7.44$, $p < .05$). Overall, rifampicin resistance was significantly higher than isoniazid resistance (64% vs 50%), with specimens 1.75 times more likely to be resistant to rifampicin ($\chi^2 = 12.20$, $p = 0.00048$; Fisher's Exact $p = 0.00047$), highlighting the prominence of rifampicin resistance in the region. **Conclusion:** MDR-TB in the North West Province was unevenly distributed, with the highest burden in major catchments such as Tshepong and Moses Kotane, while smaller or remote facilities exhibited relatively higher MDR prevalence, indicating localized transmission hotspots. Rifampicin resistance, driven by *rpoB* mutations, was significantly higher than isoniazid resistance, suggesting faster emergence. These findings highlights the need for routine drug susceptibility testing, targeted treatment, and adherence monitoring to limit MDR-TB spread and improve outcomes

Keywords: Tuberculosis (TB),Multidrug-resistant TB (MDR-TB), Rifampicin resistance, Isoniazid resistance,*rpoB, katG, inhA* mutations, Drug susceptibility,North West Province

INTRODUCTION

Tuberculosis (TB) is an ancient human disease, with *Mycobacterium tuberculosis* as the causative organism (Emegano *et al*.,2025). Tuberculosis (TB) remains a major public health challenge, especially in low- and middle-income countries (Arora *et al.*,2025).TB most frequently affects the respiratory system however, it might have adverse effects on different organs in the body (Emegano *et al*.,2025).TB transmission is airborne, through tiny

droplets emanating from coughs or sneezes from the carrier (Emegano *et al*.,2025).

Isoniazid (INH) is a key first-line drug and a crucial component of TB treatment owing to its rapid bactericidal activity (Maleki,2025). INH plays a significant role in reducing the bacterial load in sputum when combined with other anti-TB drugs in sensitive drug regimens. (Maleki,2025).During the first two weeks of treatment, the bacterial count decreased by 10- to 100-fold (1–2 log)(Maleki,2025). Rifampicin, or rifampin, is a frontline antibiotic used in a multidrug regimen as part of standard chemotherapy to treat TB disease, and acquisition of Rifampicin-Resistant Determining Region (RDR) is a prerequisite to multi-drug resistance, with the WHO estimating numbers of Drug-Resistant TB(DR TB) cases without distinguishing RDR and MDR (Bobba and Khader,2023). The mechanism of action for rifampicin is through binding to the core of the multi-subunit DNA-dependent RNA polymerase machinery and preventing elongation of RNA transcripts (Bobba and Khader,2023). Specifically, the antibiotic binds in a pocket of *rpoB* and bacterial resistance to rifampicin in bacteria is mediated primarily through mutations in a section of *rpoB* known as the rifampicin resistance determining region (RRDR) (Bobba and Khader,2023).Multidrug-resistant TB (MDR-TB) occurs when bacteria become resistant to primary TB medications, specifically rifampicin (RIF) and isoniazid (INH) (Singh *et al*.,2025).

Analysing TB and MDR-TB distribution in the North West Province helps reveal how the disease spreads and identifies areas that contribute most to the overall TB burden. Examining facility trends and resistance profiles helps identify transmission hotspots and gaps in diagnosis or treatment. Understanding key resistance mutations (*rpoB, katG, inhA*) shows whether resistance is transmitted or acquired, supporting better resource allocation, targeted case finding, and strengthened TB services.

Literature review

Annually, the number of TB prevalent cases is approximately 10 million people globally with more than one million deaths attributed to TB (Kibuuka *et al*.,2021).Most of these deaths are in low-income countries (Kibuuka *et al*.,2021). South Africa remains one of the most TB burdened countries with an estimated annual TB incidence of 301,000 cases in 2018(Kibuuka *et al*.,2021). In 2019, the World Health Organisation estimated the mortality rate of all forms of TB for South Africa, excluding deaths among those coinfected with TB/HIV, to be 37 and 73 per 100,000 population, respectively (Kibuuka *et al*.,2021).

Mechanisms of Drug Resistance in Mycobacterium tuberculosis

There are various mechanisms by which drug resistance in bacteria occurs(Biswas *et al.,*2021) . Bacterial drug resistance may be intrinsic or acquired (Biswas *et al*.,2021). Intrinsic drug resistance mechanisms occur by genes specified for resistance present in the host DNA, e.g., β lactamase gene of Gram-negative bacteria(Biswas *et al*.,2021). Mechanisms of acquired drug resistance include mutations in antibiotic-targeted genes and the transfer of resistance factors present on plasmids, transposons, bacteriophages, and other mobile genetic elements via transduction, conjugation, and transformation (Biswas *et al*.,2021).

According to relevant studies at home and abroad, the mutation of the rifampin resistance determining region (RRDR) in the *rpoB* gene of MTB is closely related to 95% of rifampicin-resistant cases (7–9). (Zhu *et al*.,2025) Rifampicin cannot be firmly bound to the β subunit of DNA-dependent RNA polymerase, and cannot inhibit the synthesis of mycobacterium tuberculosis RNA, so it loses its bactericidal effect(Zhu *et al*.,2025). The molecular basis of INH resistance is mutations in the *katG, inhA*, or *inhA* promoter genes of Mtb, to which about 80% of its phenotypic resistance in the world is attributed (Bakhtiyariniya *et al.*,2022) . INH is a prodrug that diffuses passively into growing bacilli(Bakhtiyariniya *et al.,*2022). The enzyme catalase-peroxidase (*KatG*), encoded by the *katG* gene, converts INH to the active form to exert its lethal effect by inhibiting mycolic acid biosynthesis, an essential component of the mycobacterial cell wall(Bakhtiyariniya *et al*.,2022). The enzyme InhA, or enoyl-acyl carrier protein (ACP) reductase, is encoded by the inhA gene and involved in mycolic acid biosynthesis(Bakhtiyariniya *et al*.,2022).Activated INH binds to and inhibits this enzyme (Bakhtiyariniya *et al.,*2022).

Clinical and Treatment Implications of Rifampicin and Isoniazid Resistance

In 2021, global analyses confirmed that RIF resistance significantly increases the risk of treatment failure and mortality because it eliminates one of the most potent sterilising agents in the TB regimen (Global Tuberculosis Report, 2021). Loss of RIF activity results in prolonged bacterial persistence, delayed sputum conversion and increased infectiousness, especially in high-burden settings (Zhang *et al*., 2021).

Treatment implications differ between the two drugs (Valafar,2021). Patients with RIF resistance require immediate transition to MDR-TB regimens (Garcia-Basteiro *et al.,* 2021). In contrast, INH resistance alone does not require MDR-TB treatment but necessitates modification of first-line therapy (Valafar,2021). Failure to adjust treatment appropriately leads to amplification of resistance, highlighting the importance of early detection (Valafar,2021).When RIF and INH resistance occur

together, forming MDR-TB, the clinical consequences become more severe (Garcia-Basteiro *et al*., 2021).

Overall, evidence from 2021 literature shows that RIF and INH resistance significantly impact treatment outcomes, patient prognosis and public health efforts (Van Deun *et al.,* 2021). Strengthening molecular diagnostic capacity, ensuring rapid initiation of appropriate regimens and improving patient support systems remain critical for reducing morbidity and preventing further spread of resistant TB strains (Garcia-Basteiro *et al.,* 2021).

Behavioural Patterns of RIF and INH Resistance

INH resistance shows a heterogeneous mutation pattern, mainly involving *katG* and *inhA* promoter mutations (Valafar,2021). These mutations vary by region and may be missed by routine molecular tests, causing diagnostic gaps and ongoing transmission (Valafar, 2021). RIF resistance is dominated by *rpoB* mutations, but borderline/low-level mutations in the RRDR often escape detection or appear susceptible in some tests, yet still cause poor clinical outcomes when untreated as resistant (Van Deun *et al*., 2021). Another important behaviour is heteroresistance, where both resistant and susceptible populations coexist within the same patient(Tamilzhalagan *et al*., 2021).This leads to inconsistent test results and risk of selecting fully resistant strains during treatment (Tamilzhalagan *et al.,* 2021). Studies in 2021 also highlighted compensatory mutations in genes such as *rpoA* and rpoC, which restore fitness to RIF-resistant strains, enabling them to transmit as effectively as drug-susceptible TB. This explains the persistence and spread of highly successful MDR-TB lineages globally (Ma *et al.,* 2021).

Methodology

Study location

This retrospective study was conducted using data from the Tshepong Hospital TB Referral Laboratory in Klerksdorp, North West Province**.**

Research design

This study was a retrospective, quantitative investigation that utilized existing data obtained from the National Health Laboratory Service (NHLS) Central Data Warehouse (CDW). A systematic analysis was conducted on quantifiable data, and appropriate statistical techniques were applied to evaluate drug sensitivity profiling.

Population and sampling

Patient details and laboratory episode numbers were not included in the dataset. The data included information on the instruments used to perform the tests, results for first-line drugs, and the referring facilities. Approval to access the data was obtained from the NHLS through the AARMS application, and ethical clearance was granted by the HSREC prior to submission to the NHLS for final approval. All NHLS laboratory data are stored in the Laboratory Information System (LIS) and are centrally managed through the CDW for data extraction. A stratified randomized dataset was selected for this study.

Lab analysis methodology

The sputum samples were processed according to National Health Laboratory Service TB Laboratory Standard Operating Procedure (SOP) and according to Good Laboratory Practice (GLP). Personal Protective Equipment were used during sample processing. The samples were processed inside the Biosafety Cabinet level 2 by qualified and competent staff members. SOPs used to process the samples were as follows: MIC0462 version 10,MIC1425 version 6, GPL3695 for GeneXpert processing Operation, Maintenance and Troubleshooting; GPL4487 version 2 for GeneXpert/XDR and GPL4510 version 1.

Sputum samples were decontaminated using the NALC-NaOH method, centrifuged, and inoculated into MGIT liquid culture tubes supplemented with PANTA. Smears were prepared, stained with Auramine O, and examined under a fluorescent microscope for acid-fast bacilli (AFB). AFB-positive samples were tested for rifampicin resistance using Xpert® MTB/RIF Ultra and, if resistant, further analysed with GeneXpert® MTB/XDR for first- and second-line drug susceptibility. Positive MGIT cultures were differentiated between Mycobacterium tuberculosis complex (MTBC) and non-tuberculous mycobacteria (NTM) using smear staining and TB antigen assays or Line Probe Assays. Confirmed TB-positive samples were re-tested for rifampicin resistance. For molecular testing, BD MAX™ MDR-TB Sample Tubes were prepared with treated sputum, processed, and loaded into the BD MAX™ Instrument, with results recorded in the Laboratory Information System and stored in the CDW.

Data collection

This study analysed 340 Mycobacterium tuberculosis-positive isolates collected between May 2023 and May 2025. Data were obtained from the NHLS Academic Affairs and Research Management System (AARMS) and the Central Data Warehouse (CDW), with patient identities excluded for confidentiality. Laboratory results included first- and second-line drug susceptibility. Data were presented using graphs, charts, and tables, and statistical analyses Chi-square or Fisher's exact test for RIF and INH resistance, and one-way ANOVA for gene melting temperatures—were performed to profile drug-resistance patterns by gene, antibiotic, age group, and referring facility.

Data Analysis

Data were analysed using Microsoft Excel. Frequencies and percentages were calculated for sensitivity and resistance. Chi-square and Fisher's exact test were applied to assess associations between resistance patterns and demographic variables.

Results

Table 1: Distribution and prevalence of MDR-TB by facility.

Facilities (Catchment Areas)	DSTB Cases	DSTB (%)	MDR TB Cases	MDR TB (%)	Total TB Cases	Prevalence (%) TB Cases
Tshepong Hospital (Catchment Areas)	10	6.5	95	61.3	155	45.6
Other / Small Facilities	6	10.7	29	51.8	56	16.5
Moses Kotane Hospital Catchment Areas	2	4.4	32	71.1	45	13.2
Rustenburg Hospital Catchment Areas	0	0.0	12	75.0	16	4.7
Mafikeng Hospital Catchment Area	0	0.0	8	50.0	16	4.7
Gelukspan Catchment (Community Hospital)	1	6.7	10	66.7	15	4.4
Nic Bodenstein Catchment Areas	1	8.3	6	50.0	12	3.5
Schweizer Reneke Hospital Catchment Areas	0	0.0	3	33.3	9	2.6
Swartruggens Hospital Catchment Areas	0	0.0	5	83.3	6	1.8
Lehurutshe Catchment Areas	0	0.0	3	75.0	4	1.2
Bloemhof/Christiana Catchment Area	0	0.0	1	33.3	3	0.9
Lichtenburg Catchment Area	0	0.0	2	100.0	2	0.6
Joe Morolong Memorial Hospital	0	0.0	1	100.0	1	0.3
Grand Total	20	5.9	207	60.9	340	100

Table 1 indicates the distribution and prevalence of MDR-TB based on the facilities or Catchment areas. The study shows that the Tshepong Catchment area reported 155 cases (45.6%), MDR-TB accounted for 61% (n = 95). The Moses Kotane Catchment areas were second with the highest cases (n = cases; 13.2%) with an MDR prevalence of 71.1% (n = 32) .Other small facilities combined showed n = 56 cases with a prevalence of 16.5%. The Rustenburg and Mafikeng Catchment areas contributed 4.7% each (n =16); MDR cases for the Rustenburg Catchment area were 75% (n = 12) and 8 for the Mafikeng Catchment area with a prevalence of 50%. The Gelukspan Catchment area contributed 4.4% (n = 15) of cases with a high MDR prevalence of 66.7%. The Nic Bodenstein Catchment contributed 3.5% (n = 12). The Swartruggens, Lehurutshe, Schweizer Reneke, Bloemhof/Christiana, and Lichtenburg Catchment areas had small numbers of cases (<3% each), but several had an MDR prevalence ≥ 75%.The Joe Morolong Memorial Hospital reported only one case

(0.3% prevalence) which was the smallest of all, but it was 100% MDR-TB.

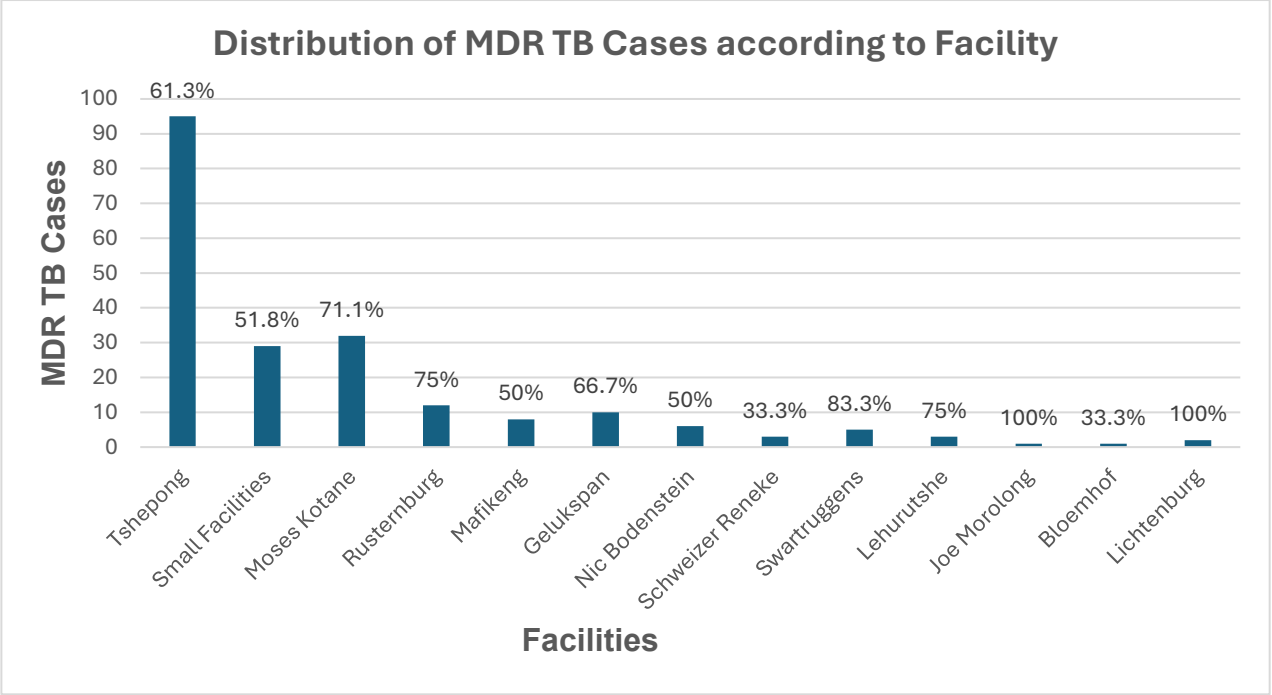

Figure 1: Distribution and Prevalence of MDR-TB Cases Reported Across Health Facilities in the North West Province.

Figure 1 shows that Tshepong Hospital reported 95 cases of MDR-TB with the prevalence of 61.3% followed by Moses Kotane Hospital with 32 cases and the prevalence of 51.8%. All other facilities contributed much smaller numbers of MDR-TB cases.

Table 2: Genetic Mutation associated with First line drugs Resistance in Mycobacterium tuberculosis

Genes	Drug affected	Resistant isolates(n)	Resistant isolates (%)
rpoB	**Rifampicin**	**219**	**64.4**
KatG	**Isoniazid**	**173**	**50.9**
inhA	**Isoniazid**	**173**	**50.9**

Table 2 show that the *rpoB* gene for Rifampicin resistance was the gene with the highest resistant isolates (n = 219; 64.4%). The *KatG* gene for Isoniazid resistance was the second gene after *rpoB* with the highest resistant isolates (n = 173; 50%).

Table 3:Chi square results

Test	χ^2	df	p-value(approx)
Goodness-of-Fit (distribution of gene mutations)	**7.44**	**2**	**~0.024**

Table 3 indicates chi square results which shows that the distribution of resistant isolates differed significantly across *rpoB*, *katG*, and *inhA* genes,

$\chi^2(2) = 7.44$, $p < .05$.

This shows that resistance related to *rpoB* (64.4%), indicating rifampicin resistance arises more prominently than isoniazid-associated mutations.

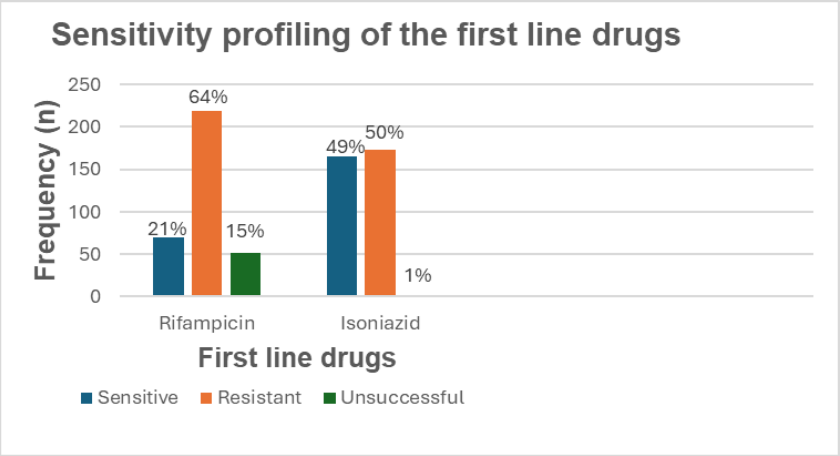

Figure 2: Sensitive and resistant outcomes for the Rifampicin and the Isoniazid.

Figure 2 shows that out of the 340 specimens tested, 64% (n = 219) were resistant to rifampicin, while only 21% (n = 70) were sensitive. A significant proportion (15%; n = 52) produced. Fifty per cent (50%) (n = 173) of isolates were resistant to isoniazid with 49% (n = 165) sensitive and 1% (n = 2) unsuccessful. The resistance rate for RIF was significantly higher than for INH (64% vs 50%).

Table 4: Comparison of Resistance Between Rifampicin and Isoniazid

Drug	Resistant (n)	Non-Resistant (n)
Rifampicin	**219**	**121**
Isoniazid	**173**	**167**

Table 4 indicates the comparison of resistance between rifampicin and isoniazid, which shows that frequency (n) = 219 of rifampicin which are resistant and n = 173 of isoniazid which are resistant. The resistance rates for rifampicin (RIF) and isoniazid (INH) were compared using a 2×2 contingency table. The combined number of sensitive and unsuccessful results were considered non-resistant. Chi-square test and Fisher's Exact Test were used to compare the resistance between the Rifampicin and Isoniazid. Chi-square test yielded $\chi^2 = 12.20$, $p = 0.00048$, indicating a statistically significant difference in resistance rates between RIF and INH. Fisher's Exact Test confirmed this result with $p = 0.00047$ and an odds ratio of 1.75, indicating that specimens were 1.75 times more likely to be resistant to RIF compared to INH.

Discussion

This study analzed the distribution and MDR patterns in the North West Province behavioural insights into rifampicin and isoniazid resistance and their treatment

implications at the Tshepong NHLS Referral TB Laboratory in the North West Province of South Africa.

In the North West Province, MDR-TB cases were unevenly distributed across catchment areas, with the highest burden observed in Tshepong (n = 155, 45.6% of cases; MDR prevalence 61%) and Moses Kotane (n = 32, 13.2%; MDR prevalence 71.1%). Smaller catchment areas, including Rustenburg, Mafikeng, Gelukspan, and Nic Bodenstein, contributed fewer cases but often exhibited high MDR-TB prevalence, ranging from 50% to 75%. Several minor facilities (<3% of cases each) reported very high MDR rates (≥75%), and even isolated cases, such as the single patient at Joe Morolong Memorial Hospital, were MDR-TB. Overall, the pattern indicates that while MDR-TB is concentrated in larger hospitals, smaller or remote catchments display disproportionately high MDR prevalence, suggesting localized transmission hotspots across the province. The *rpoB* gene for Rifampicin resistance was the gene with the highest resistant isolates (n = 219; 64.4%). The *KatG* gene for Isoniazid resistance was the second gene after *rpoB* with the highest resistant isolates (n = 173; 50%).A chi-square goodness-of-fit test was performed to determine whether resistance-associated mutations were evenly distributed among *rpoB, katG*, and *inhA*. The distribution differed significantly from an equal pattern,

$\chi^2(2) = 7.44, p < .05.$

The results showed that the mutations in *rpoB* occurred more frequently (64.4%) than expected, indicating rifampicin resistance is disproportionately higher compared to isoniazid resistance mutations (*katG* and *inhA*, both 50.9%). This suggests that rifampicin resistance mechanisms may develop more prominently—behaviorally or evolutionarily—than isoniazid resistance mechanisms.

The comparison of resistance patterns between rifampicin (RIF) and isoniazid (INH) revealed that rifampicin resistance was significantly higher (n = 219) than isoniazid resistance (n = 173). Statistical analysis using chi-square (χ^2 = 12.20, p = 0.00048) and Fisher's Exact Test (p = 0.00047) confirmed this difference, with an odds ratio of 1.75, indicating specimens were 1.75 times more likely to be resistant to RIF than INH. This higher expected resistance suggests that rifampicin resistance may emerge more rapidly, potentially due to behavioural factors such as incomplete treatment adherence, drug misuse, or pharmacokinetic differences. Clinically, the findings highlights the importance of routine drug susceptibility testing to guide individualized therapy, strengthen adherence monitoring, and optimize treatment regimens to prevent the further emergence and spread of MDR-TB.

Conclusion

In the North West Province, MDR-TB reveals a variable distribution , with the highest burden concentrated in major catchment areas such as Tshepong and Moses Kotane, while smaller or remote facilities display relatively higher high MDR prevalence, highlighting potential localized transmission hotspots. Rifampicin resistance, driven primarily by *rpoB* mutations, was significantly higher than isoniazid resistance, suggesting that rifampicin resistance may emerge more rapidly due to behavioural, evolutionary, or treatment-related factors. These findings emphasize the critical need for routine drug susceptibility testing, individualized treatment regimens, and strengthened adherence monitoring to limit the spread of MDR-TB and improve patient outcomes across both high- and low-burden settings in the province.

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Conflict of interest: None

Financial support: None

Ethical clearance

Permission to use NHLS data was granted under AARMS reference number PR2455151, and ethical approval was obtained from the Health Sciences Research Ethics Committee (HSREC). All patient information was deidentified, kept confidential, and protected according to POPIA and the National Health Act. Only essential data for the study were provided by the NHLS, and no additional investigations were conducted. Data were used

solely for research purposes, with strict adherence to NHLS policies and confidentiality requirements.

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