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Article

From Molecular Surveillance to Governance Intelligence: Insights from Routine Drug-Resistance Mutation Proxies in Rural Eastern Cape

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Abstract

Background/Objectives: Drug-resistant tuberculosis (TB) remains a major challenge in high-burden settings, where timely identification of emerging resistance and effective governance responses are critical. While routine molecular diagnostics generate large volumes of resistance-associated mutation data, these outputs are typically used for individual patient management and remain underutilized for population-level surveillance and for the application of clinical governance approaches for improved TB care. **Methods:** We conducted a retrospective cross-sectional analysis of 1,386 molecular diagnostic records for *Mycobacterium tuberculosis*, collected between March 2021 and December 2024, from 30 health facilities in the K.S.D Local Municipality of O.R. Tambo District. Resistance-associated mutation proxies were identified for loci associated with isoniazid (*katG*, *inhA*), fluoroquinolone (*gyrA*), and second-line injectable agents (amikacin, kanamycin, and capreomycin) through mutations in the *rrs* locus. Mutation proxy prevalence was examined overall, by age group, over time, and across facilities. Persistence of resistance detection was assessed using consecutive-month analyses to characterize temporal continuity at the facility level. **Results:** At least one resistance-associated mutation proxy was detected in 72.7% of tests. Isoniazid-associated mutation proxies predominated, with *katG* detected in 52.2% and *inhA* in 20.2% of records, while fluoroquinolone- and injectable-associated proxies were less frequent. Resistance-associated mutation proxies were observed across all adult age groups, with the highest burden and greatest resistance diversity among individuals aged 25–44 years. Substantial temporal variation was evident, including declining annual prevalence for most mutation proxies between 2022 and 2024, alongside increasing *inhA* prevalence. Marked facility-level heterogeneity was observed, with high-volume referral sites contributing the largest absolute burden of resistant cases. Prolonged persistence of mutation detection, including uninterrupted runs of up to 15 months, was identified at selected facilities. **Conclusions:** Routine molecular diagnostic data revealed a substantial and heterogeneous burden of drug-resistant *Mycobacterium tuberculosis* in K.S.D. Local Municipality, characterized by age-specific patterns, temporal shifts, and sustained facility-level persistence. Beyond descriptive epidemiology, routinely generated mutation proxy data can serve as early-warning indicators of clinical governance stress, signaling emerging pressures on TB care systems when resistance patterns persist or worsen. Interpreting these trends can support more anticipatory clinical governance, strengthen resistance surveillance, and guide prioritized interventions in high-burden, resource-constrained settings.

Keywords: drug-resistant tuberculosis; molecular diagnostics; mutation proxies; clinical governance; surveillance; isoniazid resistance; fluoroquinolone resistance

1. Introduction

Tuberculosis (TB) remains a leading cause of morbidity and mortality globally, with drug-resistant forms posing a persistent threat to TB control efforts [1,2]. South Africa is among the countries with the highest burden of both drug-sensitive and drug-resistant TB, where resistance to first-line and second-line anti-TB drugs complicates treatment and undermines programmatic outcomes [3,4]. Understanding the distribution and patterns of drug resistance is therefore critical for informing clinical management at the individual patient level and public health response strategies at the health system level.

The widespread implementation of molecular diagnostic assays has transformed TB diagnosis by enabling rapid detection of *Mycobacterium tuberculosis* and key resistance-associated mutations [5,6]. These assays generate routine data on resistance-associated genetic changes at loci such as *katG*, *inhA*, *gyrA*, and *rrs*, which are commonly used as proxies for resistance to isoniazid, fluoroquinolones, and injectable agents. While such mutation proxy data are primarily used to guide individual patient management, they also represent an underutilized resource for describing population-level patterns of drug resistance and for informing policy and practice guidelines.

Most epidemiological studies of drug-resistant TB rely on culture-based drug susceptibility testing or whole-genome sequencing, approaches that may be limited by cost, turnaround time, and incomplete coverage in resource-constrained settings [7]. In contrast, routinely generated molecular diagnostic data are widely available and reflect resistance patterns encountered in everyday clinical practice. However, these data are often under-analysed beyond aggregate reporting, and few studies have systematically examined variation in resistance-associated mutation proxies by age, time, and health facility.

In high-burden districts such as Oliver Reginald (O.R.) In the Eastern Cape Province, healthcare access, referral pathways, and patient populations vary substantially across facilities. As a result, aggregated resistance estimates may obscure important heterogeneity patterns within the burden of drug-resistant TB. Disaggregated analyses that consider temporal trends, age-specific patterns, and facility-level variation are therefore essential for a more nuanced understanding of resistance dynamics.

Beyond descriptive epidemiology, these findings highlight the potential to repurpose routinely generated mutation proxy data as early-warning indicators of clinical governance stress, which are situations in which healthcare systems or facilities experience strain in maintaining the quality, safety, and effectiveness of clinical care due to emerging risks, increasing service pressure, or gaps in oversight and response mechanisms. Such stress typically becomes evident when routine clinical or diagnostic indicators begin to signal persistent or worsening disease patterns that require coordinated clinical, managerial, and programmatic responses. Interpreting mutation proxy trends through this governance lens can support more anticipatory clinical governance, strengthen oversight of TB resistance diagnostics, and enable prioritized interventions in high-burden, resource-constrained settings.

This study aimed to describe the prevalence and distribution of resistance-associated mutation proxies in *Mycobacterium tuberculosis* using routine molecular diagnostic data collected over 4 years in the King Sabatha Dalindyebo (K.S.D.) local municipality in the O.R. Tambo District, Eastern Cape, South Africa. Specifically, we examined overall and gene-specific mutation proxy prevalence, temporal variation, age-stratified patterns, facility-level heterogeneity, and the persistence of resistance detection over time. By leveraging routinely collected diagnostic data, this study provides real-world insights into patterns of drug-resistant TB in a high-burden setting.

2. Materials and Methods

2.1. Study Design and Data Source

A retrospective cross-sectional analysis was conducted using laboratory routinely generated molecular tuberculosis diagnostic data collected from health facilities in K.S.D. Local Municipality (KSDLM), O.R. Tambo District, Eastern Cape, South Africa, between March 2021 and December 2024.

2.2. Study Setting and Population

KSD Local Municipality, a category B municipality, is located within the O.R. Tambo District in the Eastern Cape Province. It is the largest of the five municipalities in the district, accounting for a quarter of its geographical area. The municipality is the largest in terms of surface area among the five (5) local municipalities within the district, covering approximately 3,027 km² spread over 37 Wards. KSD is one of the 257 local municipalities in South Africa. The estimated population in KSD is 476,558, with 114,580 households. KSDLM is predominantly a rural municipality with approximately 95% of households living in rural areas. Regarding gender, 54% of the population is female, and 46% is male. Most of the population is young. The main towns are Mqanduli and Mthatha (formerly Umtata), and the key economic sectors include community services, finance, and trade [KSDM, 2025]. KSDLM is predominantly African, and IsiXhosa is the most widely spoken language. The area is characterized by a high burden of TB and HIV, limited healthcare access in rural settlements, and persistent social inequalities affecting health-seeking behavior. Figure 1 shows KSDLM. All *Mycobacterium tuberculosis*-positive molecular diagnostic records generated during the study period were eligible for inclusion. A total of 1,386 records from 30 health facilities were analyzed. Available variables included test date, facility, patient age, and probe outputs for resistance-associated mutations. Gender data were unavailable and were not analyzed.

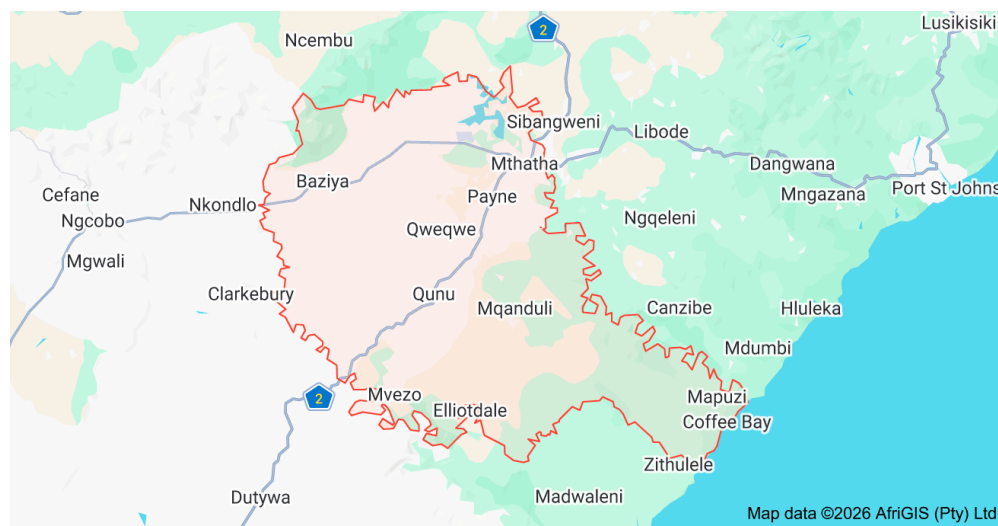


Figure 1. Map showing the KSD local municipality.

2.3. Definition of Resistance-Associated Mutation Proxies

Resistance-associated mutation proxies were defined using mutant melt peak temperature signals generated by WHO-endorsed molecular diagnostic assays (Alcántara et al., 2020). A mutation proxy was classified as present when a mutant melt peak was detected for a resistance-associated locus and absent when no such signal was observed. Proxies were defined for *katG* and *inhA* as indicators of isoniazid resistance, *gyrA* (any region) as an indicator of fluoroquinolone resistance, *rrs* as an indicator of resistance to injectable agents, and *eis* as an indicator of kanamycin resistance. In line with the WHO SMART guideline terminology [9,10], molecular assays are designed to detect

“mutations associated with resistance to anti-tuberculosis medicines”. They are intended to support “routine surveillance of drug resistance using data generated through diagnostic services.” Accordingly, these mutation proxies are interpreted as assay-derived molecular indicators rather than sequencing-confirmed mutations. Their use is appropriate for population-level surveillance and clinical governance of TB management, as they are generated routinely during standard care, enable timely identification of emerging resistance patterns, and provide actionable intelligence to inform facility-level monitoring, prioritization, and early clinical governance intervention to improve clinical outcomes in high-burden settings.

2.4. Outcomes and Stratification

The primary outcome was the prevalence of resistance-associated mutation proxies, defined as the proportion of tests with at least one detected proxy. Secondary analyses examined gene-specific prevalence, monthly and annual variation, age-stratified distribution, facility-level variation, and persistence of mutation detection. Age was categorized into standard epidemiological groups: <15, 15–24, 25–34, 35–44, 45–54, and ≥55 years.

2.5. Temporal and Facility-Level Analyses

Monthly and annual prevalence estimates were calculated using the test date. Facility-level analyses were restricted to facilities with ≥10 tests to ensure stable estimates. Facilities were assigned anonymized codes for reporting. Persistence of resistance detection was assessed by identifying consecutive months with at least one mutation-positive test at each facility. A separate analysis was conducted for *gyrA* mutation proxies.

2.6. Statistical Analysis

Data was analysed using Microsoft Excel 365 (version 16.x; Microsoft Corporation, Redmond, WA, USA) for data cleaning and preliminary summaries. Descriptive statistical analyses, temporal aggregation, and data visualization were conducted using Python (version 3.10; Python Software Foundation, Wilmington, DE, USA), with the pandas library (version 2.1; USA) for data manipulation, NumPy (version 1.26; USA) for numerical operations, and matplotlib (version 3.8; USA) for graphical visualization.

2.7. Ethical Considerations

The study utilized de-identified secondary laboratory data generated through routine diagnostic services. Ethical clearance was obtained from the Research Ethics and Biosafety Committee, Faculty of Health Sciences, Walter Sisulu University (Ref: WSU HREC 141/2025; approved 2 July 2025). The Eastern Cape Department of Health granted administrative permission (Ref: EC_202507_023; approved 11 July 2025). National Health Laboratory Services granted the collection of laboratory diagnostic patient sample results data (Ref: SR4169693 approved 25 November 2025).

3. Results

3.1 Diagnostic Volume and Dataset Characteristics

A total of 1,386 molecular diagnostic records were analysed, collected between March 2021 and December 2024, from 30 health facilities within KSDLM. Diagnostic volume increased substantially over time. The majority of tests were performed in 2024 (976/1,386; 70.4%), followed by 2023 (308/1,386; 22.2%), 2022 (73/1,386; 5.3%), and 2021 (29/1,386; 2.1%).

3.2. Overall Prevalence of Drug-Resistance Mutation Proxies

Across all records, 1,007 of 1,386 tests (72.7%) demonstrated at least one resistance-associated mutation proxy (Table 1). Isoniazid-associated mutation proxies were most frequently detected, with

katG mutations identified in 724 tests (52.2%) and *inhA* mutations in 280 tests (20.2%). Second-line resistance-associated mutation proxies were less common.

Table 1. Overall prevalence of resistance-associated mutation proxies.

Resistance-associated locus	Mutation proxy detected, n (%)	Total tests
Any mutation proxy	1,007 (72.7)	1,386
<i>katG</i>	724 (52.2)	1,386
<i>inhA</i>	280 (20.2)	1,386
<i>gyrA</i> (any region)	149 (10.8)	1,386
<i>rrs</i>	125 (9.0)	1,386
<i>eis</i>	0 (0.0)	1,386

3.3. Temporal Patterns in Drug-Resistance Mutation Proxies

3.3.1. Monthly Variation

Figure 2 below demonstrates marked month-to-month variability in resistance-associated mutation proxy prevalence, against a consistently high background prevalence of mutation proxies detected by molecular diagnostic tests. The prevalence of any mutation proxy remained elevated throughout the study period, peaking in October 2024, when 86.0% (92/107) of tests were mutation-positive, indicating sustained high levels of possible drug-resistant *Mycobacterium tuberculosis*. Isoniazid resistance, reflected by *katG* mutation proxies, showed persistent but fluctuating prevalence, with a prominent peak in November 2023 (63.7%; 58/91), suggesting episodic intensification rather than a uniform temporal trend. In contrast, second-line resistance proxies displayed more episodic behaviour, with sharp, temporally clustered peaks in September 2023 for both *gyrA* (41.4%; 12/29) and *rrs* (34.5%; 10/29), indicating periods of increased presentation or detection of more complex resistance patterns. The *inhA* mutation proxies remained lower overall but increased later in the study period, consistent with a shifting resistance profile over time (Figure 2). Overall, the figure highlights a pattern of sustained baseline resistance punctuated by discrete temporal surges, particularly for second-line resistance markers, underscoring the importance of longitudinal monitoring, as short-term peaks may signal emerging resistance pressure or delayed clinical and programmatic responses that require timely review.

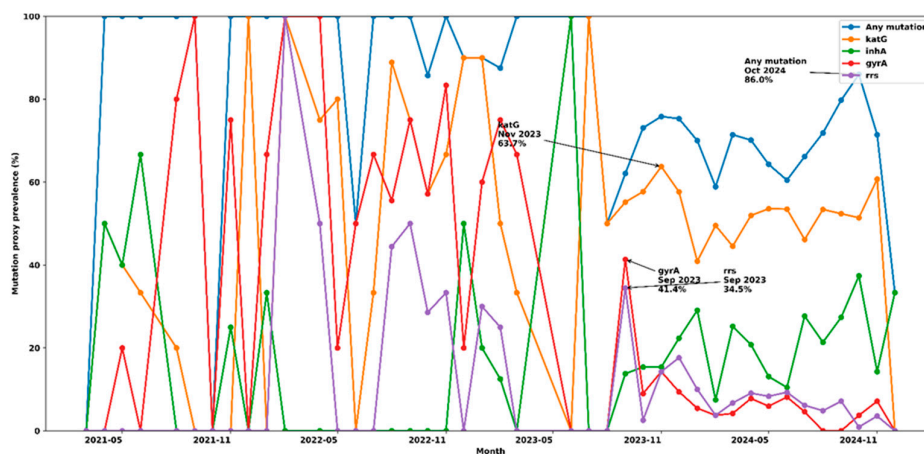


Figure 2. Monthly variation in resistance-associated mutation proxy prevalence.

3.3.2. Annual Variation

Annual aggregation demonstrated clear temporal shifts in the prevalence of resistance-associated mutation proxies, as indicated (Table 1). The prevalence of any mutation proxy declined progressively from 95.9% (70/73) in 2022 to 74.7% (230/308) in 2023, and then to 70.0% (684/976) in 2024. Gene-specific trends showed divergent patterns over time. The prevalence of *katG* mutation proxies decreased steadily from 68.5% in 2022 to 59.7% in 2023 and 49.5% in 2024, while *inhA* mutation proxies increased from 8.2% to 17.2% and further to 22.0% over the same period. In contrast, second-line resistance proxies declined markedly, with *gyrA* mutation prevalence falling from 57.5% in 2022 to 17.5% in 2023 and 4.3% in 2024, and *rrs* mutation proxies decreasing from 24.7% to 14.6% and 6.4%, respectively.

3.3.2.1. Annual Variation in Resistance-Associated Mutation Proxy Prevalence

Figure 3 below illustrates changes in the annual prevalence of resistance-associated mutation proxies between 2022 and 2024. The prevalence of any mutation proxy declined over the study period, decreasing from 95.9% in 2022 to 74.7% in 2023 and 70.0% in 2024. Gene-specific trends varied across resistance loci. The prevalence of *katG* mutation proxies decreased progressively from 68.5% in 2022 to 59.7% in 2023 and 49.5% in 2024. In contrast, *inhA* mutation proxies increased over time, rising from 8.2% in 2022 to 17.2% in 2023 and 22.0% in 2024. For second-line resistance proxies, the prevalence of *gyrA* mutations declined markedly from 57.5% in 2022 to 17.5% in 2023 and 4.3% in 2024, while *rrs* mutation proxies decreased from 24.7% to 14.6% and 6.4% over the same period.

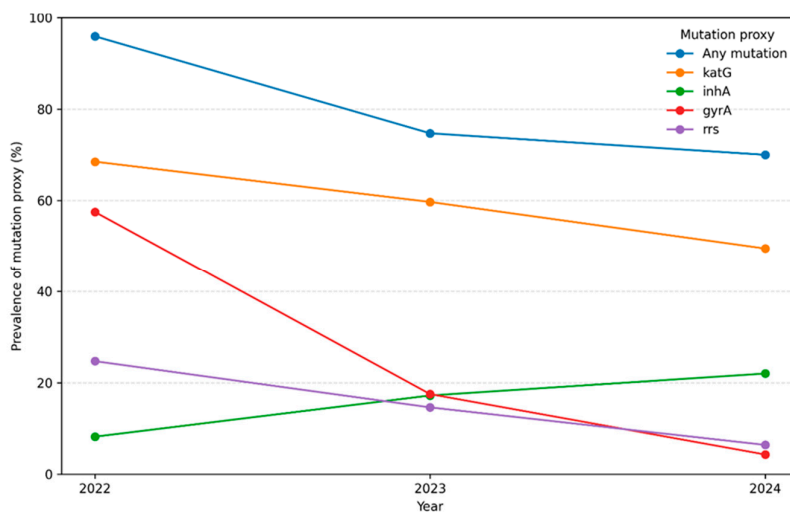


Figure 3. Annual prevalence of resistance-associated mutation proxies between 2022 and 2024. Lines represent the proportion of molecular diagnostic tests with detected mutation proxies for any resistance, *katG*, *inhA*, *gyrA*, and *rrs*.

3.4. Age-Stratified Distribution of Drug-Resistance Mutation Proxies

Resistance-associated mutation proxies were detected across all adult age groups (Table 2). The 15–24-year age group demonstrated the highest prevalence of any mutation proxy, with 153 of 157 tests (97.5%) positive, predominantly due to *katG* mutations. The 25–34-year and 35–44-year age groups contributed the largest absolute numbers of mutation-positive tests and showed the greatest diversity of resistance-associated mutation proxies, including substantial detection of *gyrA* and *rrs* mutations.

Table 2. Age-stratified prevalence of resistance-associated mutation proxies.

Age group (years)	Tests, n	Any mutation n (%)	<i>katG</i> n (%)	<i>inhA</i> n (%)	<i>gyrA</i> n (%)	<i>rrs</i> n (%)
<15	4	0 (0.0)	0	0	0	0
15–24	157	153 (97.5)	129 (82.2)	24 (15.3)	1 (0.6)	0 (0.0)
25–34	322	231 (71.7)	128 (39.8)	60 (18.6)	89 (27.6)	34 (10.6)
35–44	389	265 (68.1)	155 (39.8)	134 (34.4)	17 (4.4)	46 (11.8)
45–54	244	149 (61.1)	103 (42.2)	46 (18.9)	0 (0.0)	3 (1.2)
≥55	270	209 (77.4)	209 (77.4)	16 (5.9)	42 (15.6)	42 (15.6)

3.5. Facility-Level Distribution of Drug-Resistance Mutation Proxies

Facility (F)-level analysis revealed marked heterogeneity in the prevalence and distribution of resistance-associated mutation proxies across health facilities. Several sites exhibited extremely high mutation proxy prevalence, including F1 (16/16; 100%), F2 (13/13; 100%), and F3 (71/75; 94.7%), although these estimates were influenced by small diagnostic volumes at some facilities. In contrast, F4 contributed the largest absolute number of mutation-positive tests (589/699; 84.3%), reflecting its substantially higher diagnostic throughput rather than disproportionately elevated prevalence alone. When prevalence and diagnostic volume were considered jointly, substantial inter-facility heterogeneity became evident, with some facilities characterized by very high percentage positivity but low testing volume, and others contributing a high absolute burden of resistant cases due to large service volumes.

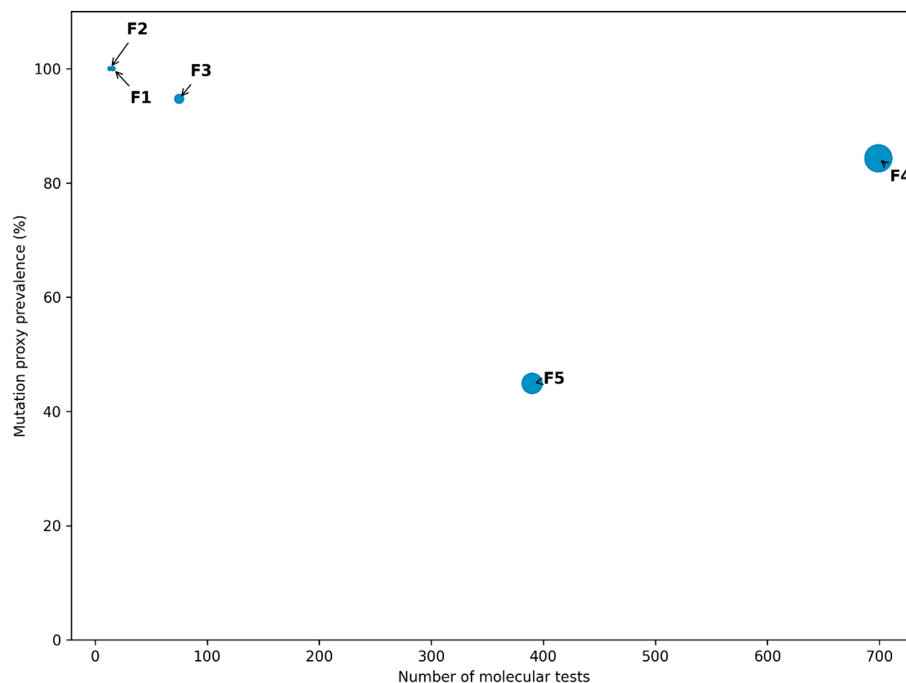


Figure 4. Facility-level distribution of resistance-associated mutation proxy prevalence and diagnostic volume.

3.6. Persistence of Drug-Resistance Detection Over Time

Consecutive months with mutation-positive tests were observed at selected facilities (Figure 5). F4 demonstrated mutation-positive results in 29 of 46 months, with a longest continuous run of 15 months. F2 demonstrated mutation-positive results in 19 of 36 months, also with a longest continuous run of 15 months. Other facilities showed shorter continuous runs of mutation detection, ranging from 2 to 5 months. Analysis restricted to fluoroquinolone-associated *gyrA* mutation proxies showed prolonged recurrence at F4, with *gyrA* mutations detected in 27 months and uninterrupted runs of up to 14 months.

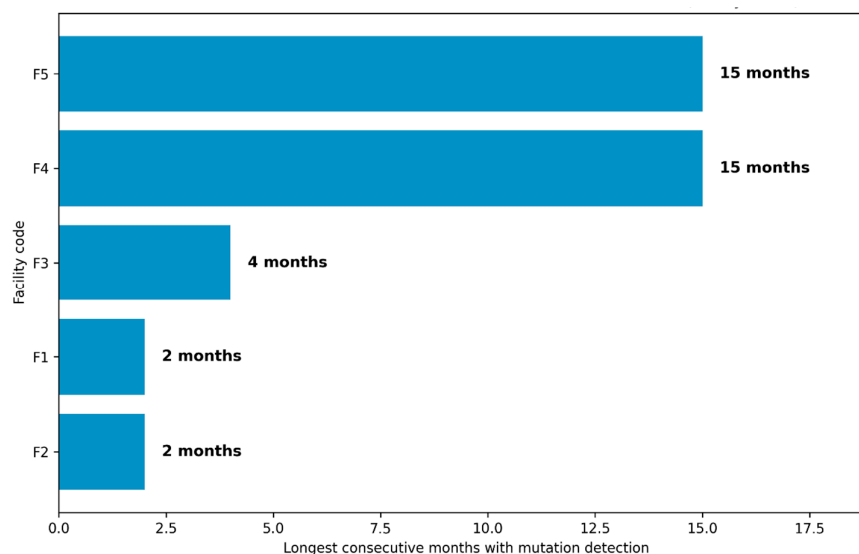


Figure 5. Duration of the longest uninterrupted period of resistance-associated mutation detection at selected health facilities.

The Facility-Level Governance Priority Score (GPS) conceptual model (Figure 6) integrates four routinely available indicators: resistance-associated mutation proxy prevalence, mutation diversity across *katG*, *inhA*, *gyrA*, and *rrs* loci, persistence of mutation detection over consecutive months, and diagnostic volume to stratify facilities into governance-critical, governance-watch, and governance-stable categories. This framework reframes facility-level heterogeneity from a descriptive epidemiological finding into a decision-support tool for prioritizing anticipatory clinical governance interventions to enhance the response to emerging patterns of drug resistance. Clinical governance, in this instance, would entail analyzing and monitoring resistance patterns against clinical outcomes trends to determine the clinical significance of these mutation proxy trends for current TB management approaches.

Application of the GPS framework to selected high-impact facilities demonstrated clear differentiation in governance risk profiles (Table 3). Facilities F2 and F4 were classified as governance-critical, reflecting the convergence of resistance complexity, including substantial second-line resistance, and, in the case of F4, high diagnostic volume with prolonged persistence of mutation detection. In contrast, facilities F1, F3, and F5 were classified as governance-watch facilities, characterized by high proportional resistance prevalence or notable second-line signals but lower diagnostic volume, referral-driven case mix, or limited evidence of sustained persistence. No facilities within this subset met the criteria for governance-stable classification.

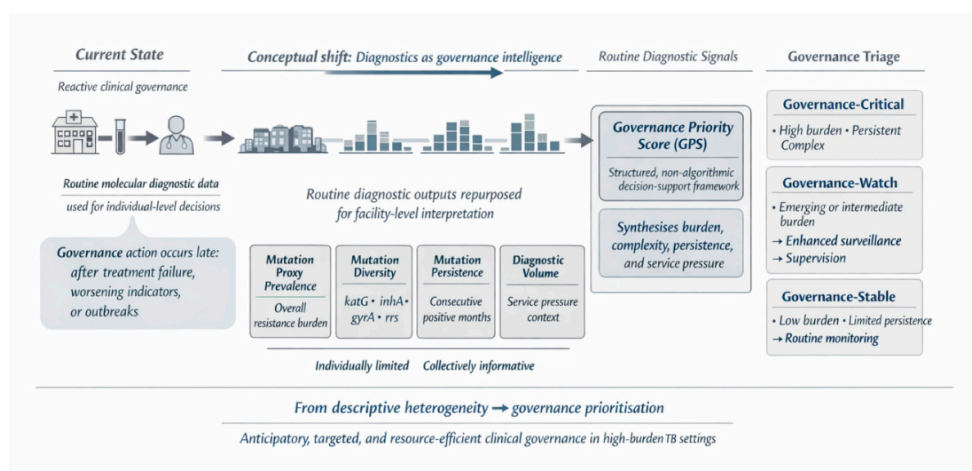


Figure 6. Facility-level governance triage model based on routine molecular diagnostic data.

Table 3. Governance triage classification of selected high-impact facilities (F1–F5).

Facility code	Diagnostic volume (n)	Any mutation proxy (%)	Key resistance features	Persistence/volume context	Governance triage category	Rationale for classification
F1	16	16 (100%)	Predominantly first-line resistance; no <i>gyrA</i> or <i>rrs</i> detected	Very low volume; no documented persistence	Governance-Watch	Extremely high proportional prevalence based on small numbers; requires monitoring

						rather than immediate intensive intervention.
F2	13	13 (100%)	High second-line resistance burden (<i>gyrA</i> 69.2%, <i>rrs</i> 46.2%)	Low volume but high resistance complexity	Governance-Critical	Extensive fluoroquinolone and injectable resistance signals indicate elevated governance risk.
F3	75	71 (94.7%)	Resistance is largely limited to first-line drugs	Moderate volume; referral-level case mix	Governance-Watch	High prevalence expected in a tertiary referral setting; patterns reflect case complexity
F4	699	589 (84.3%)	Significant second-line resistance (<i>gyrA</i> 17.5%, <i>rrs</i> 11.7%)	Very high volume; prolonged persistence (up to 15 months)	Governance-Critical	Convergence of high absolute burden, resistance diversity, and persistence indicates sustained governance stress.
F5	13	11 (84.6%)	Prominent fluoroquinolone resistance (<i>gyrA</i> 46.2%)	Low-moderate volume; no RRs detected	Governance-Watch	High proportional resistance to the second-line signal warrants enhanced surveillance.

4. Discussion

4.1. Diagnostic Volume and Dataset Maturity

Our study analysed 1,386 molecular diagnostic records collected between March 2021 and December 2024 across 30 health facilities in KSDLM, in the O.R. Tambo District Municipality of the Eastern Cape, South Africa. Diagnostic volume increased markedly over time, with more than 70% of tests performed in 2024, reflecting the progressive expansion and routine integration of molecular diagnostics into district TB services. Earlier years contributed smaller sample sizes and likely reflect a more selective testing phase focused on higher-risk patients. This pattern may also have been influenced by disruptions to routine health services during the COVID-19 pandemic, which affected TB case detection, diagnostic testing volumes, and healthcare access in many high-burden settings [11]. Nevertheless, the multi-year span of the dataset provides a valuable longitudinal perspective, enabling assessment of evolving drug-resistance patterns as diagnostic coverage matured within routine clinical practice.

4.2. Burden of Drug-Resistance Mutation Proxies

The predominance of *katG*-associated mutation proxies in this study is consistent with global and regional evidence showing that mutations in *katG*, particularly codon 315, are the dominant mechanism of isoniazid resistance in *Mycobacterium tuberculosis* [12]. African molecular epidemiology studies have similarly reported persistent transmission and evolution of drug-resistant TB strains, with resistance dynamics influenced by treatment pressures and population-level factors such as HIV co-infection [13]. These findings reinforce the value of routinely generated molecular diagnostic data as surveillance tools, enabling early detection of emerging resistance signals and supporting anticipatory clinical governance and strengthened programmatic TB resistance monitoring in high-burden settings. The concurrent detection of *inhA* mutation proxies (20.2%) in this study indicates the coexistence of multiple mechanisms of isoniazid resistance within the same population. This pattern is consistent with findings from several African settings where *katG* mutations, typically associated with high-level isoniazid resistance, coexist with *inhA* promoter mutations that confer lower-level resistance through target overexpression [14]. Similar distributions of *katG* and *inhA* mutations have been reported in studies from Cameroon and other African regions, highlighting the heterogeneous molecular landscape of isoniazid resistance across the continent [12,14]. Research conducted in the Eastern Cape has further demonstrated that undetected isoniazid monoresistance, particularly linked to *inhA* promoter mutations, represents a critical pathway for the emergence of multidrug-resistant tuberculosis, especially when routine diagnostic algorithms prioritise detection of rifampicin resistance [15]. Comparable mutation patterns have also been observed in studies from Uganda and Ghana, where *katG* S315T remains the dominant mutation while *inhA* promoter mutations contribute substantially to resistance diversity [12,13].

In contrast, the relatively low detection of fluoroquinolone-associated (*gyrA*) and injectable-associated (*rrs*) mutation proxies, together with the absence of *eis* mutations, is consistent with the well-described stepwise accumulation of drug resistance in *Mycobacterium tuberculosis*, where resistance to first-line drugs typically precedes the emergence of resistance to second-line agents. This pattern reflects the prolonged historical use of first-line drugs such as isoniazid and rifampicin in TB treatment programs, which creates sustained selective pressure favouring mutations in genes such as *katG* and *inhA*. In contrast, mutations conferring resistance to second-line agents remain comparatively rare. Similar findings have been reported in studies from Ethiopia and other high-burden settings, where resistance to first-line drugs substantially exceeds resistance to fluoroquinolones or injectable agents [16]. In that study, resistance to any second-line drug was detected in only 5.6% of isolates, including a small proportion of pre-XDR and extensively drug-resistant (XDR) cases, highlighting the comparatively limited spread of second-line resistance despite widespread first-line resistance [16].

This gradient in resistance acquisition is also supported by epidemiological and modelling studies demonstrating that resistance typically emerges through sequential genetic adaptation during inadequate or interrupted treatment, with first-line resistance creating a pathway for the subsequent accumulation of additional mutations leading to multidrug-resistant (MDR), pre-XDR, and XDR-TB. Mathematical modelling frameworks further illustrate how the transition from drug-susceptible infection to first-line resistance, and eventually to second-line resistance, can occur through progressive selection under treatment pressure, reinforcing the concept that first-line resistance serves as the initial gateway in the evolutionary trajectory toward more complex resistance profiles [17].

From a clinical governance and surveillance perspective, this pattern has important implications. The predominance of first-line resistance markers, alongside relatively limited second-line resistance, suggests a critical window for intervention, in which strengthened diagnostic surveillance, early detection of isoniazid and rifampicin resistance, and rapid optimisation of treatment regimens could prevent further escalation toward fluoroquinolone and injectable resistance. Monitoring mutation proxies across key resistance loci therefore provides not only molecular epidemiological insight but also a governance-relevant early signal of resistance evolution, enabling health systems to prioritise facilities or patient populations that require intensified diagnostic oversight and treatment stewardship.

4.3. Temporal Patterns in Drug-Resistance Prevalence

Marked temporal variation in the prevalence of resistance-associated mutation proxies observed in this study is consistent with reports from other molecular epidemiology studies showing that drug-resistance mutations in *Mycobacterium tuberculosis* do not emerge uniformly over time but fluctuate according to transmission dynamics, treatment pressures, and diagnostic practices. In our dataset, distinct temporal peaks were observed across resistance loci: *gyrA* and *rrs* mutation proxies peaked in 2023, and *inhA* increased later in 2024, indicating shifting resistance profiles across drug classes. Similar heterogeneity in mutation distribution has been described in other studies, where resistance-associated mutations vary over time and across populations as selective pressure from treatment regimens changes. These findings support the concept that drug resistance evolves dynamically and is influenced by both programmatic and epidemiological factors, rather than following a single linear trend [18]. In our dataset, sustained baseline detection of resistance markers punctuated by short-term peaks suggests episodic amplification of resistant strains or localized transmission events. Such temporal fluctuations underscore the importance of longitudinal surveillance using routinely generated molecular diagnostic data, as short-term increases in specific mutation proxies may signal emerging resistance patterns that require programmatic review and clinical governance intervention. Annual aggregation showed a decline in overall mutation proxy prevalence between 2022 and 2024, although this trend should be interpreted cautiously. The early years of the study coincided with residual disruptions from the COVID-19 pandemic, which reduced TB service access and diagnostic activity in many settings, often limiting testing to patients with severe disease or high clinical suspicion. As TB services recovered and diagnostic capacity expanded in subsequent years, testing likely included a broader and more representative clinical population. Consequently, the observed decline in proportional prevalence may reflect changes in testing patterns rather than a true reduction in drug-resistant tuberculosis, despite an increase in the absolute number of detected cases.

4.4. Age-Stratified Distribution of Drug-Resistance Mutation Proxies

Resistance-associated mutation proxies were detected across all adult age groups, with notable variation in both prevalence and resistance profiles. A higher burden of resistance-associated mutations among adults has important implications for TB management, as this age group represents the most socially and economically active segment of the population and therefore plays a key role in ongoing transmission of drug-resistant *Mycobacterium tuberculosis*. Adults are also more likely

to have prior TB treatment exposure, treatment interruption, or delayed diagnosis, factors that contribute to the development and amplification of drug resistance. Consequently, the predominance of resistance markers in adults highlights the need for rapid molecular resistance testing, prompt initiation of appropriate second-line regimens, and strengthened adherence support to prevent further transmission and progression to more complex resistance patterns [14,19,20].

Higher mutation-proxy prevalence in adults (especially in economically active ages) usually reflects where TB transmission and diagnosis concentrate: adults have higher social mixing and workplace/community exposure, and they also account for the bulk of TB case detection, so resistance markers are more often observed and have greater programmatic impact. In African datasets, *katG/inhA* patterns also differ by age, with *katG* frequently predominating across the economically active group, reinforcing the need for age-targeted adherence support and contact-tracing intensity where resistant TB circulates [14]. Our findings of very high prevalence in 15–24 years, dominated by *katG* with minimal second-line resistance, are more consistent with ongoing transmission of isoniazid-resistant strains than with stepwise acquisition during repeated treatment episodes. This interpretation is biologically plausible because *katG* Ser315Thr is a high-frequency, “low fitness cost” mutation that can be transmitted efficiently [21] and is also widely reported as the dominant INH-resistance mutation in African and global summaries [12].

Adolescents and young adults represent a highly mobile population, and the concentration of *katG*-associated resistance in this group is consistent with ongoing community-level spread of first-line drug-resistant strains [14,22]. Importantly, the relative absence of second-line resistance proxies indicates that fluoroquinolones and injectable agents remain largely preserved, highlighting a critical window for early intervention. In contrast, a laboratory-based surveillance study on second-line resistance among MDR patients in Ethiopia revealed that although the majority of MDR isolates included in the study were susceptible to second-line treatment (94.02%), a small proportion (6%) of the isolates were resistant to at least one of the second-line drugs, most likely among new TB cases. The study further reported the occurrence as acquired resistance due to treatment failure, leading to late resistance emergence [23]. These findings underscore the importance of routine upfront molecular drug-susceptibility testing in younger patients to ensure timely regimen optimization and prevent resistance amplification. From a programmatic perspective, age-stratified molecular surveillance can serve as an early warning signal, enabling targeted prevention, intensified case-finding, and youth-focused adherence and engagement strategies to interrupt transmission before progression to more complex, drug-resistant tuberculosis patterns.

In contrast, the 25–34 and 35–44-year age groups contributed the largest absolute numbers of mutation-positive tests and exhibited the greatest diversity of resistance patterns, including notable detection of *gyrA* and *rrs*. These age groups comprise the most socioeconomically active part of the population. Individuals aged 25 to 44 have higher mobility, greater occupational exposure, and larger social networks, all of which facilitate sustained community transmission. The high prevalence of mutation-positive tuberculosis in this demographic shows that transmission occurs among economically productive, socially connected groups, perhaps maintaining transmission chains at the community level. Moreover, the diversity of resistance patterns to fluoroquinolones and second-line injectables may suggest the distribution of strains with more complex resistance profiles. This may be attributed to prior incomplete or inadequate treatment, transmission of already drug-resistant strains, and ongoing selective pressure from ongoing fluoroquinolone use for the treatment of other infections. Furthermore, the concentration of various resistance mutations in young-to-middle adulthood raises concerns about the long-term transmission of pre-XDR strains among highly interacting population groupings. If these people are not recognized or treated properly, they may become reservoirs for resistant tuberculosis, increasing the likelihood of future progression to XDR-TB.

Among individuals aged ≥ 55 years, resistance remained dominated by *katG* mutations, with lower prevalence of *inhA* mutations but persistent detection of second-line resistance proxies. These findings are in collaboration with a study by Charan et al., which documented the most common

mutation in INH monoresistance as *katG* (65.1%) and a lower prevalence of *inhA* resistance (28.1%) [24]. Conversely, a study on age-independent resistance in India reported lower mutation frequencies among older patients, suggesting that this may be attributed to the reactivation of older, drug-susceptible infections [25]. No resistance-associated mutation proxies were identified among the small number of tests from individuals under 15 years of age. Collectively, these findings indicate that drug-resistant TB affects adults across the lifespan, with peak burden and complexity concentrated in economically active age groups, consistent with cumulative exposure and prior treatment history. Comparable resistance profiles have been described in studies conducted in South Africa, Nigeria, Iran, and Thailand [26–29]. In contrast, a study focusing on the rural context of the TB burden reported the highest peak of TB incidence in those aged >70, suggesting causality to be associated with chronic disease, subsequently predisposing old age to TB [30].

4.5. Facility-Level Heterogeneity in Drug-Resistance Burden

Substantial heterogeneity in resistance burden was observed across health facilities, indicating that drug-resistant *Mycobacterium tuberculosis* is unevenly distributed, with certain facilities likely functioning as focal points of sustained transmission and resistance amplification rather than reflecting a uniform district-wide phenomenon. Such variability is consistent with differences in local transmission dynamics, patient case-mix, referral patterns, and service delivery factors, and highlights the limitations of relying on aggregated program-level indicators to guide treatment decisions [31,32].

Clinically, patients presenting to high-burden facilities face an increased risk of initial regimen mismatch if standardised approaches are applied without timely molecular drug-susceptibility testing, potentially leading to early treatment failure and prolonged infectiousness [33]. Within a facility-level governance and early-warning framework, this heterogeneity becomes actionable intelligence: routine aggregation of molecular resistance signals enables early identification of facilities requiring intensified diagnostic coverage, clinical oversight, adherence support, and infection prevention measures, thereby supporting anticipatory, targeted interventions to contain resistance before progression to more complex drug-resistant TB patterns. Several sites demonstrated extremely high mutation proxy prevalence, including facilities with 100% positivity among tested samples, although small denominators strongly influence such estimates. In contrast, F4 contributed the largest absolute number of mutation-positive records, reflecting its high diagnostic throughput rather than solely its disproportionately high prevalence. These findings underscore the importance of interpreting facility-level resistance prevalence in conjunction with diagnostic volume to avoid overestimating resistance burden in low-volume facilities or underestimating the contribution of high-volume referral centres.

4.6. Persistence of Resistance Detection over Time

The prolonged persistence of resistance-associated mutation detection at selected facilities, most notably F2 and F4, indicates that drug-resistant *Mycobacterium tuberculosis* is being encountered continuously within these catchment areas rather than arising from sporadic or isolated cases. Sustained mutation positivity over periods of up to 15 months suggests ongoing transmission, recurrent presentation of resistant disease, or persistent programmatic gaps, such as delayed diagnosis, suboptimal regimen matching, or challenges with treatment adherence and continuity of care [34]. The particularly prolonged recurrence of fluoroquinolone-associated *gyrA* mutation proxies at F4, extending over 27 months with uninterrupted runs of up to 14 months, is of great clinical and programmatic concern, as it signals sustained second-line resistance pressure in a high-burden setting. From a treatment perspective, this threatens the effectiveness of cornerstone MDR-TB regimens and underscores the need for early, routine molecular drug-susceptibility testing and heightened clinical oversight at affected facilities [35]. Within a facility-level governance and early-warning framework, such persistence should be interpreted as a red-flag indicator warranting prioritized intervention, including intensified diagnostic coverage, regimen review, adherence

support, and infection prevention measures, to prevent further amplification and entrenchment of second-line drug-resistant tuberculosis.

4.7. Mutation Persistence as a Proxy Indicator of Clinical Governance Stress

Conventional clinical governance frameworks in TB programs are largely reactive, responding to treatment failure, poor adherence, or established drug resistance after adverse outcomes have already occurred [36]. Findings from this study support an alternative, predictive framing in which routinely generated molecular diagnostic data are repurposed as early-warning indicators of governance stress from a clinical perspective at the facility level. The monitoring and use of drug resistance trends could be critical in ensuring evidence-based decision-making at individual facilities to minimize the risks of treatment failure, poor clinical outcomes, and possible progression to severe forms of drug resistance.

Facility-level persistence analyses revealed prolonged, uninterrupted periods of proxy detection for resistance-associated mutations at selected sites, with continuous runs lasting up to 15 months. Sustained mutation positivity over time, particularly when observed across multiple resistance-associated loci, likely reflects more than ongoing transmission or complex case-mix alone. Instead, such patterns plausibly indicate weaknesses in upstream clinical governance processes, including delayed regimen optimization, inconsistent clinical guideline review and updating, suboptimal adherence support, or gaps in treatment outcomes monitoring.

Within this framework, mutation persistence is conceptualized not solely as a microbiological phenomenon but also as a proxy indicator of strains, potential strains, and possible weaknesses in the governance of healthcare services. We propose operationalizing a Mutation Persistence Threshold as the detection of one or more resistance-associated mutation proxies for six or more consecutive months at a facility. Exceeding this threshold signals accumulated, unmet health services governance and oversight needs and should prompt structured interventions to address community and facility factors to address the emerging trend.

Clinical Governance responses triggered by sustained persistence may include targeted clinical audits of drug-resistant TB cases, intensified adherence support and patient tracing, and multidisciplinary regimen review, particularly in facilities demonstrating persistent fluoroquinolone-associated mutation proxies. Importantly, this approach requires no additional laboratory testing, novel diagnostics, or complex modelling. Instead, it transforms existing routine molecular outputs into actionable clinical governance intelligence to improve the quality of TB services.

By shifting clinical governance from retrospective oversight to anticipatory intervention, persistence-based metrics enable earlier identification of facilities at risk of sustained drug-resistant TB burden. This predictive framing aligns with data-informed health-system strengthening priorities. It provides a practical pathway for integrating laboratory surveillance into routine care and for improving workflow in TB in high-burden, resource-constrained settings.

4.8. Facility-Level Governance Triage Using a Governance Priority Score

Most analyses of drug-resistant TB focus on identifying where resistance prevalence is highest. While epidemiologically informative, such descriptions offer limited guidance on how to prioritize scarce health system resources across heterogeneous facilities to improve TB care. In this study, we adopt a systems-oriented inversion, reframing the analytic question from where resistance is high to where clinical governance intervention is most urgently required.

To support this reframing, we propose a Facility-Level Governance Priority Score (GPS) that integrates four dimensions derived entirely from routine molecular diagnostic data: (i) facility-level prevalence of resistance-associated mutation proxies; (ii) mutation diversity across key resistance loci (*katG*, *inhA*, *gyrA*, *rrs*); (iii) persistence of mutation detection over consecutive months; and (iv) diagnostic volume.

This composite perspective recognizes that facilities with moderate prevalence, but prolonged persistence or high diagnostic throughput, may contribute disproportionately to the ongoing drug-

resistant TB burden. Conversely, facilities with very high prevalence estimates derived from small test numbers may warrant closer monitoring rather than immediate intensive intervention. The GPS, therefore, moves beyond prevalence alone, incorporating temporal continuity, resistance complexity, and service pressure.

Using this framework, facilities can be conceptually stratified into three clinical governance priority categories: governance-critical facilities exhibiting convergence of high prevalence, multi-locus diversity, prolonged persistence, and substantial volume; governance-watch facilities demonstrating intermediate or emerging burden; and governance-stable facilities characterized by low prevalence, limited diversity, and minimal persistence. The GPS is not intended as a rigid scoring algorithm but as a pragmatic decision-support tool to guide proportional clinical governance responses, including clinical audits, feedback to patient-facing clinicians, adherence interventions, review of clinical guidelines and regimens, and supervisory support.

By translating routine molecular surveillance outputs into a structured clinical governance triage model, this approach strengthens the linkage between laboratory data and health-system optimization, enabling more efficient and anticipatory responses to drug-resistant TB.

4.9. Facility-Level Governance Triage Model Based on Routine Molecular Diagnostic Data

In current practice, molecular diagnostic data are predominantly used as patient-level clinical tools, with governance actions typically triggered only after treatment failure, deteriorating program indicators, or recognized outbreaks. This downstream, reactive configuration limits health systems' capacity to respond proactively to emerging patterns of drug-resistant TB.

The proposed Facility-Level Governance Triage Model reorients the use of routine diagnostic data toward anticipatory, system-level intelligence. By aggregating mutation proxy data at the facility level, resistance signals can cluster and acquire operational meaning beyond individual patient management. Rather than focusing exclusively on prevalence, the model identifies facilities where clinical governance intervention is most urgently required.

Four routinely available indicators, mutation proxy prevalence, mutation diversity, mutation persistence, and diagnostic volume, are jointly interpreted to provide a multidimensional assessment of resistance burden, complexity, temporal continuity, and service demand. Individually, each indicator offers limited insight; collectively, they inform prioritization of governance intensity.

These indicators are synthesized within the GPS framework to stratify facilities into governance-critical, governance-watch, and governance-stable categories, each linked to proportionate governance actions ranging from targeted audits and diagnostic and treatment regimen reviews to enhanced surveillance and clinical outcomes monitoring. The model thus reframes facility-level heterogeneity from a descriptive epidemiological observation into an operational governance tool.

4.10. Temporal Lag Between Resistance Detection and Clinical Governance Response

A recurring challenge in the performance of TB programs is the temporal disconnect between early resistance detection and an appropriate clinical governance response to tailor diagnostic and treatment approaches. While molecular diagnostics enable rapid identification of resistance-associated mutations, governance actions such as intensified supervision or program-level intervention are often initiated only after sustained adverse outcomes become apparent. This suggests that health system governance systems may lack an effective and responsive mechanism for measuring, monitoring, and responding to early warning signals of gaps and weaknesses in program performance.

Building on persistence analyses, we introduce the concept of a Clinical Governance Lag Index, defined as the interval between the initial detection of resistance-associated mutation proxies, their persistence over time, and the eventual escalation to prompt an appropriate clinical governance response. In this framework, prolonged uninterrupted runs of mutation-positive months represent accumulated, unmet governance signals rather than isolated microbiological events.

Facilities demonstrating continuous mutation detection for 12–15 months may therefore be conceptualized as governance-lag hotspots, where early resistance signals were repeatedly detected but not translated into timely intervention. In such settings, persistence likely reflects both ongoing transmission and complex case-mix, as well as a delayed or insufficient clinical governance response.

Recognizing governance lag as a system-level phenomenon reframes mutation persistence as an indicator of temporal misalignment between surveillance and action. Embedding predefined persistence thresholds into routine program oversight could strengthen governance memory, reduce delays in intervention, and shorten the duration during which drug-resistant TB remains unaddressed at the facility level. This temporal perspective reinforces the role of routine molecular surveillance as an early-warning system and supports more responsive, anticipatory clinical governance.

4.11. Strengths and Limitations

Strengths

This study leverages a large, multi-year dataset of routinely generated molecular diagnostic records to capture real-world patterns of drug resistance across multiple health facilities in a high-burden district. Because mutation proxy data were generated as part of standard clinical care, the findings are highly relevant to routine programmatic decision-making and reflect resistance patterns encountered in everyday practice rather than those observed in selected research cohorts. The inclusion of age-stratified analyses adds epidemiological depth, allowing assessment of how resistance burden and complexity vary across the adult lifespan and highlighting the population groups that contribute most to the observed resistance patterns.

Limitations

Several limitations should be acknowledged. Resistance was inferred using assay-derived mutation proxies rather than sequencing-confirmed mutations, which may not capture all resistance mechanisms or distinguish between specific genetic variants. Diagnostic practices and testing volume evolved as molecular diagnostics were progressively scaled up, potentially influencing the observed temporal trends. As with all analyses based on routine surveillance data, these factors should be considered when interpreting changes in prevalence and persistence across the study period.

4.12. Recommendations

The TB programme should integrate routine monitoring of resistance-associated mutation proxies into standard clinical governance processes, with particular attention to the persistence of mutation detection across consecutive months as an early warning signal of governance stress. The adoption of predefined persistence thresholds (e.g., ≥ 6 consecutive months) can trigger timely, structured responses, including targeted clinical audits, timely feedback to clinicians, updates to clinical practice guidelines, regimen review, and intensified adherence support before adverse outcomes become entrenched. To optimize the use of limited governance resources, health systems should move beyond prevalence-only assessments and implement triage-based, multidimensional frameworks such as the GPS, which systematically link resistance burden, complexity, persistence, and diagnostic volume to prioritized action. Strengthening the operational linkage between laboratory surveillance and governance decision-making is essential to ensure that routinely generated molecular diagnostic data are translated into timely programmatic and clinical interventions. Finally, the GPS framework should be applied and validated in other high-burden settings to assess its generalizability and potential impact on treatment outcomes, resistance amplification, and overall program performance.

5. Conclusions

This study demonstrates that routinely generated molecular diagnostic data contain underutilized signals that are highly relevant for clinical governance beyond individual patient management. Analysis of resistance-associated mutation proxies revealed a substantial and heterogeneous burden of drug-resistant *Mycobacterium tuberculosis* in KSDLM in O.R. Tambo District, characterized by persistent isoniazid resistance, episodic emergence of second-line resistance, and marked facility-level variability. The identification of prolonged mutation persistence at selected facilities underscores sustained resistance pressure that conventional, outcome-triggered governance approaches may not capture.

The proposed Facility-Level GPS reframes routine molecular surveillance data as a pragmatic decision-support tool for anticipatory clinical governance by integrating resistance burden, complexity, persistence, and diagnostic volume. Its application demonstrated clear differentiation between facilities requiring urgent governance intervention and those warranting enhanced surveillance. By repurposing existing diagnostic data into actionable governance intelligence, this approach offers a feasible pathway to reduce governance lag and strengthen timely responses to drug-resistant tuberculosis in high-burden, resource-constrained settings.

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Abbreviations

The following abbreviations are used in this manuscript:

F	Facility
GPS	Governance Priority Score
KSD	King Sabatha Dalindyebo
KSDLM	King Sabatha Dalindyebo Local Municipality
MDR	Multidrug resistant
O.R	Oliver Reginald
TB	Tuberculosis
XDR	Extensively drug-resistant

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