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Genetic Patterns of Mutations Associated with Drug Resistance in *Mycobacterium tuberculosis* Isolates from Culture-Positive Patients in the Republic of Congo

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Abstract

Tuberculosis (TB) remains a major public health challenge, particularly in sub-Saharan Africa, where the emergence and spread of drug-resistant *Mycobacterium tuberculosis* (MTB) strains threaten current control efforts. This study aimed to characterize the frequency and distribution of genetic mutations associated with resistance to rifampicin, isoniazid, ethambutol, fluoroquinolones, and second-line injectable agents in MTB isolates from culture-positive pulmonary TB patients in the Republic of Congo. A total of 45 MTB culture-positive samples collected between 2018 and 2019 were analyzed using targeted next-generation sequencing (MinION). Key resistance associated genes, including rpoB, katG, embB, gyrA, and rrs, were examined. Overall, 48% (22/45) of isolates harbored at least one mutation conferring drug resistance. Drug resistance associated mutations were detected in 48% (15/22) of sequences for rifampicin, mainly driven by Ser531Leu, Asp516Val, and His526Tyr. For Ethambutol, the prevalence was 50% (11/22), mainly associated with Met306Val mutation. Isoniazid represented 40.9% (9/22), primarily linked with Ser315Thr mutation. The floroquinolone represented 9% (2/22) mainly driven by Ala90Val and Asp94Gly. Lastly aminoglycosides resistance counted for 4% (1/22) mainly A1401G mutation. The results underscore the critical need to enhance molecular surveillance and strengthen treatment protocols for drug-resistant TB in the Republic of Congo to effectively combat this evolving health crisis.

Keywords: *Mycobacterium tuberculosis*; drug resistance; mutations; Republic of Congo

1. Introduction

Tuberculosis (TB) remains a major infectious disease and public health concern worldwide, with an estimated 10.8 million new cases and 1.25 million deaths in 2024 [1]. The emergence and spread of drug-resistant TB (DR-TB) has further complicated global control strategies. Multidrug-resistant TB (MDR-TB), which represents a resistance to the two most potent anti-TB drugs (isoniazid and rifampicin) and extensively drug-resistant TB (XDR-TB), defined as MDR/RR with additional resistant to any fluoroquinolone (FLQ) and at least one additional group A drug (currently bedaquiline or linezolid) pose a growing threat to TB elimination goals [2]. In the same year, approximately 400,000 individuals developed MDR/RR-TB, yet only 44% received appropriate

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treatment, and the global treatment success rate remained around 60%, highlighting substantial gaps in case detection and management [1]. In the Republic of Congo (RoC), a country of 6.6 million people, TB remains endemic with an incidence of 368 per 100,000. A major concern is the estimated 560 cases of MDR/RR-TB, which have a treatment success rate of only 45% [3,4]. This is exacerbated by several factors, including widespread co-infections [5–7]. Despite the national TB program's efforts to roll out molecular diagnostics, DOT, and a sample transport network, the response is hampered by limited resources, logistical hurdles, and a critical lack of phenotypic DST capacity.

Data on specific mutations in key resistance-associated genes including rpoB, katG, gyrA, embB, and rrs are scarce in the RoC. (Table S1) This knowledge gap directly impedes clinical decision-making and undermines the development of effective public health interventions for MDR-TB control. The urgency of addressing this gap is underscored by the severe global burden of drug-resistant TB (DR-TB), which is characterized by higher mortality, prolonged treatment, and increased drug toxicity compared to drug-susceptible disease [3,8]. This information gap undermines national efforts to align with WHO's End TB Strategy, which emphasizes universal drug susceptibility testing (DST) and rapid molecular diagnostics as core components of effective TB control. Globally, drug-resistant TB (DR-TB) continues to threaten progress, being associated with higher mortality rates, longer and more toxic treatment regimens, increased treatment costs, and greater social and economic burdens compared to drug-susceptible TB.

In this context, the establishment and strengthening of in-country molecular and genomic surveillance capacity represents a critical step toward achieving national TB control objectives. The introduction of technologies such as Xpert MTB/XDR, line probe assays (LPA), and genome sequencing platforms now provide opportunities for the rapid detection of resistance-conferring mutations, characterization of circulating pathogens, and integration of genomic epidemiology into national surveillance systems in certain areas. These advances not only improve clinical management of drugs resistance cases but also enable countries to contribute to regional genomic networks and cross-border surveillance initiatives, strengthening collective capacity to combat antimicrobial resistance.

Drug resistance in *Mycobacterium tuberculosis* (MTB) results from mutations in genes encoding drug targets or activating enzymes, whose frequency varies across regions [9]. While WHO-endorsed molecular tools such as Xpert® MTB/RIF and GenoType® MTBDRplus have improved rapid detection, they identify only a limited number of known mutations, potentially missing region-specific variants. This underscores the need for expanded molecular surveillance to capture local mutation profiles [10,11].

The last molecular data from the RoC were reported by Aubry et al. (2014) using line probe assays (LPA) on isolates from Brazzaville and Pointe-Noire. That study found resistance in 46% of culture-positive samples, with identification of a double substitution (T80A–A90G) in *gyrA* causing misdetection of fluoroquinolone resistance by LPA, illustrating the assay's limitations in this setting. Since then, no updated information on resistance-conferring mutations has been generated, leaving a significant knowledge gap in national surveillance.

This study aimed to identify and describe mutations in genes associated with resistance to rifampicin, isoniazid, ethambutol, fluoroquinolones, and second-line injectable drugs in *M. tuberculosis* isolates from the Republic of the Congo using targeted gene sequencing.

2. Methods

2.1. Study Design and Sample Collection

During a cross-sectional study a total of 45 MTB GeneXpert -positive samples obtained from hospitalized Congolese TB patients screened between 2018 and 2019 at Makelekele Hospital, Brazzaville, Republic of Congo. These sample were then cultured. Briefly decontaminated sputum samples were inoculated into BACTEC MGIT 960 culture tubes (Becton Dickinson, United States of America) supplemented with oleic acid, albumin, dextrose, and catalase, together with polymyxin B,

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amphotericin B, nalidixic acid, trimethoprim, and azlocillin as antimicrobial agents. The tubes were incubated in the MGIT 960 system. Positive cultures were confirmed by Ziehl–Neelsen staining and checked for sterility on blood agar.

All confirmed MTB isolates were subsequently stored at –80°C in Middlebrook 7H9 broth supplemented with 10% OADC enrichment for downstream molecular and genomic analyses. Additional data including patient age, sex, HIV status, and treatment history were recorded anonymously.

2.2. MTBC Species Identification

Species identification within the Mycobacterium tuberculosis complex was carried out using the GenoType MTBC version 1.x assay (Hain Lifescience GmbH, Nehren, Baden-Württemberg, Germany) following the manufacturer's instructions. Briefly, genomic DNA was extracted from positive culture isolates by thermal lysis and sonication, ensuring cell disruption and release of nucleic acids. The extracted DNA served as a template for multiplex PCR amplification targeting species-specific genomic regions of MTBC members. The resulting amplicons were then subjected to reverse hybridization on nitrocellulose strips containing immobilized oligonucleotide probes specific to M. tuberculosis complex species. Hybridization and stringent washing were performed on the TWINCUBATOR® platform, and the developed strips were interpreted visually according to the manufacturer's interpretation chart.

Distinct banding patterns allowed for differentiation between major MTBC members, including *M. tuberculosis, M. africanum, M. bovis, M. microti*, and *M. caprae*, based on their unique hybridization profiles.

2.3. RNA Extraction, PCR Amplification, Sequencing, and Genome Assembly

DNA extraction from samples was performed using the conventional Chelex-100 method with slight modifications to the procedure described by Kolia-Diafouka *et al* [12]. Briefly, culture sediments were resuspended in sterile water, mixed with 10% (weight per volume) chelex-100 resin, heated at 95 °C for 15 minutes to ensure cell lysis, vortexed and centrifuged to pellet the resin and debris. The resulting supernatant containing crude DNA was collected and used as the template for downstream PCR amplification.

To ensure DNA purity and integrity, the extracted nucleic acids were quantified using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and their integrity was verified by 2% agarose gel electrophoresis and spectrophotometric analysis using a BioSpectrometer (Eppendorf, Germany), based on the A260/A280 ratio.

Drug-resistance–associated regions rpoB, katG, embB, gyrA, and rrs were amplified to assess resistance to rifampicin, isoniazid, ethambutol, fluoroquinolones, and second-line injectable drugs, respectively. The DNA concentration was quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

Amplicons were then prepared for high-throughput sequencing using Oxford Nanopores technology (ONT) platform on MinION. Briefly, the amplified genes were purified using 1xAMPure XP beads (Beckman Coulter, Brea, CA, USA) and normalized to 100ng/ μL before library preparation using ONT ligation and Native Barcoding Expansion 1–96 kit. Sequencing was performed on a MinION device for up to 24 hours. Raw reads were quality-checked using FastQC (v0.21.1) [13], and barcodes were demultiplexed and trimmed with Guppy. The processed reads were then aligned to the H37Rv reference genome (NCBI Reference Sequence NC_000962.3) using minimap2 [14], with resulting alignments sorted and indexed by SAMtools (v1.17). Alignments were visualized in IGV to inspect coverage and variant positions. Resistance-associated single nucleotide polymorphisms (SNPs) were identified using the TB-Profiler (v4) graphical user interface with default settings. Variants were further interpreted using the 2023 WHO "Catalog of mutations in the MTB complex and their association with drug resistance" [15].

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2.4. Statistical Analysis

Data analysis was performed using GraphPad Prism 8. Descriptive statistics are presented as numbers (N) with proportions (%), and continuous variables are summarized as median with interquartile range (IQR) after data distribution was assessed using the Shapiro-Wilk test. Associations between categorical variables and drug resistance were evaluated using Fisher's exact test, with a p-value < 0.05 considered statistically significant.

3. Results

The demographic and clinical characteristics of the participants and their drug resistance features are presented in Table 1. A total of 45 Mycobacterium tuberculosis complex culture-positive were analyzed. The median age of patients was 32 years (IQR 21-47), with a predominance of individuals aged >30 years. The study population included more males than females. All samples were sequenced for genetic mutations associated with drug resistance, specifically in the target genes for rifampicin (*rpoB*), isoniazid (*katG*), ethambutol (*embB*), fluoroquinolones (*gyrA*), and second-line injectables (*rrs*). Overall, 48% (22/45) of participants harbored at least one mutation associated with resistance to an antituberculosis drug. Regarding Mycobacterium species distribution, 88% of infections were caused by *M. tuberculosis* and 12% by *M. africanum*. Resistance-associated mutations were detected in 61% of M. tuberculosis isolates, whereas none of the *M. africanum* isolates carried such mutations (P < 0.05). The proportion of resistant isolates among HIV-positive participants was 80%, compared with 45% among HIV-negative participants; this difference did not reach statistical significance. The distribution of resistance by age group, sex, education level, and alcohol use is presented in Table 2.

Table 1. Thermal cycling conditions for PCR amplification of *rpoB*, *katG*, *embB*, *rrs*, and *gyrA*.

Gene	Pre-denaturation	Denaturation	Annealing	Extension	Final Extension	cycles
rpoB	94 °C, 5 min	94 °C, 30 s	59 °C, 30 s	72 °C, 1 min	72 °C, 2 min	30
katG	94 °C, 5 min	94 °C, 30 s	62 °C, 30 s	72 °C, 1 min	72 °C, 5 min	35
embB	94 °C, 3 min	94 °C, 30 s	58 °C, 30 s	72 °C, 50s	72 °C, 5 min	35
rrs	94 °C, 5 min	94 °C, 30 s	59 °C, 30 s	72 °C, 1 min	72 °C, 5 min	35
gyrA	95 °C, 5 min	95 °C, 45 s	58 °C, 45 s	72 °C, 45s	72 °C, 6 min	30

Table 2. Demographic, clinical and biological features related to drug resistance among TB patients. * Fisher's exact test.

Independent Variables Overal	Overall Sequenced (%) 45	Any drug resistance		
		Yes (%)	No (%)	P-value
Age(years) Median (IQ)	32 (21-47)	38 (21-47)	27 (20-47)	
Age group				
≤30	21 (47)	8 (38)	13 (62)	
>30	24 (53)	14 (58)	10 (42)	**0,2**
Sex				
Female	22 (31)	14 (64)	8 (36)	
Male	23 (69)	8 (35)	15 (65)	**0,07**
Education				
No schooling/Primary	7 (15)	4 (57)	3 (43)	
Secondary/University	38 (85)	12 (47)	37 (53)	**0,7**
Alcohol				

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Yes	24 (53)	14 (58)	10 (42)	**0,2**
No	21 (47)	8 (38)	13 (62)	
VIH Status				
Oui	5 (11)	4 (20)	1 (80)	**0,1**
Non	40 (89)	18 (27)	22 (73)	
MTB Species				
M. Tuberculosis	36 (88)	22 (61)	14 (39)	**0.001**
T. Africanum	5 (12)	0(0)	5 (100)	

3.1. Drug Resistance Patterns

Table 3 provides a detailed breakdown of the drug resistance profiles identified among the 22 genotypically resistant *Mycobacterium tuberculosis* complex isolates. The data reveal a high burden of resistance to first-line drugs, with concerning patterns that have direct implications for treatment regimens.

The most prominent finding was the high frequency of rifampicin resistance, which was detected in 15 out of 22 resistant isolates (68.2%; 95% CI: 48.8 – 87.6). Isoniazid and ethambutol resistance were also common, found in 9 (40.9%) and 11 (50.0%) of the resistant isolates, respectively.

Among first-line anti-tuberculosis drugs, resistance was most frequently observed in rifampicin, with 15 strains (68.2%, 95% CI: 48.8–87.6) showing mutations associated with resistance. Resistance to ethambutol was detected in 11 strains (50.0%, 95% CI: 29.1–70.9), and to isoniazid in 9 strains (40.9%, 95% CI: 20.4–61.4). Streptomycin resistance was observed in 2 strains (9.1%, 95% CI: 0.0–21.1).

For second-line drugs, resistance was less common. One strain (4.5%, 95% CI: 0.0–13.2) was resistant to kanamycin, amikacin, and capreomycin, respectively. Fluoroquinolone resistance was detected in 2 strains (9.1%, 95% CI: 0.0–21.1).

Combinations of drug resistance were also observed. Resistance to both rifampicin and isoniazid (MDR) was found in 3 strains (13.6%, 95% CI: 0.0–27.9), while 8 strains (36.4%, 95% CI: 16.2–56.5) were resistant to isoniazid and ethambutol (INH + ETB). Resistance to rifampicin, isoniazid, and ethambutol simultaneously (RIF + INH + ETB) was observed in 1 strain (4.5%, 95% CI: 0.0–13.2).

Table 3. Drug resistance profiles of 22 resistant strains.

Drug / profile	**N**	**%**	**95% CI**
First-line drugs			
Rifampicin	15	68.2	48.8 – 87.6
Isoniazid	9	40.9	20.4 – 61.4
Ethambutol	11	50.0	29.1 – 70.9
Streptomycin	2	9.1	0.0 – 21.1
Second-line drugs			
Kanamycin	1	4.5	0.0 – 13.2
Amikacin	1	4.5	0.0 – 13.2
Capreomycin	1	4.5	0.0 – 13.2
Fluoroquinolone	2	9.1	0.0 – 21.1
RIF + INH	3	13.6	0.0 – 27.9
INH + ETB	8	36.4	16.2 – 56.5
RIF + INH + ETB	1	4.5	0.0 – 13.2

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3.2. Proportion of Mutations in DR Strains

Table 4 delineates the specific genetic mutations responsible for the drug resistance observed in the 22 resistant isolates.

For rifampicin, all 15 resistant isolates had mutations in the RRDR of the rpoB gene. The most prevalent was the Ser531Leu mutation, detected in 7 isolates (46.7%), followed by Asp516Val in 4 isolates (26.7%) and His526Tyr in 2 isolates (13.3%). Other mutations included Gln513Leu and Pro535Leu, each observed in 1 isolate (6.7%), while 3 isolates (20.0%) harbored other, less common mutations.

Regarding: isoniazid resistance, the high-fitness-cost Ser315Thr mutation in the katG gene was dominant, accounting for 77.8% (7/9) of INH-resistant isolates. The remaining two isolates (22.2%) harbored the Gly279Asp mutation.

For ethambutol resistance, 11 isolates exhibited mutations in the embB gene. The Met306Val mutation was the most common, identified in 9 isolates (81.8%). Gly406Asp and Leu355Ser mutations were each detected in 1 isolate (9.1%).

Fluoroquinolone resistance was associated with mutations in the gyrA gene in 6 isolates. The Ala90Val mutation was observed in 3 isolates (50.0%), Asp94Gly in 2 isolates (33.3%), and Ser91Pro in 1 isolate (16.7%).

Finally, resistance to aminoglycosides and capreomycin was linked to mutations in the rrs gene in 3 isolates. The A1401G mutation was detected in 2 isolates (66.7%), and G1484T was observed in 1 isolate (33.3%).

Table 4. Mutational profiles of drug-resistance genes identified in *Mycobacterium tuberculosis.*

Drug	Target Gene	Observed Mutation(s)	n (%) *(among resistant isolates)*
Rifampicin (RIF)	**rpoB**		**15/22**
		Ser531Leu	7 (46.7)
		Asp516Val	4 (26.7)
		His526Tyr	2 (13.3)
		Gln513Leu	1 (6.7)
		Pro535Leu	1 (6.7)
		Other mutations	3 (20.0)
Isoniazid (INH)	**katG**		**9/22**
		Ser315Thr	7 (77.8)
		Gly279Asp	2 (22.2)
Ethambutol (EMB)	**embB**		**11/22**
		Met306Val	9 (81.8)
		Gly406Asp	1 (9.1)
		Leu355Ser	1 (9.1)
Fluoroquinolones (FQ)	**gyrA**		**6/22**
		Ala90Val	3 (50.0)
		Asp94Gly	2 (33.3)
		Ser91Pro	1 (16.7)
Aminoglycosides/Capreomycin	**rrs**		**3/22**
		A1401G	2 (66.7)
		G1484T	1 (33.3)

4. Discussion

This was the first report employing NGS to assess the frequency of genetic markers associated with first and second-line anti-TB drugs resistance in the Republic of Congo (RoC). The study provides a molecular epidemiological snapshot of drug-resistant tuberculosis in our clinical setting.

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By combining demographic data with comprehensive genetic sequencing, we have identified a high burden of drug resistance, characterized the specific mutations responsible, and uncovered significant associations with bacterial lineage.

In this study nearly half (48%) of the sequenced Mycobacterium isolates harbored at least one mutation associated with drug resistance, which was consistent with the 46% previously reported in the RoC, although that earlier study employed Line probe assays [16] . Together, these results highlight the continued burden of drug-resistant tuberculosis in this setting.

Among first-line drugs, rifampicin resistance was the most frequently detected, followed by ethambutol and isoniazid. Rifampicin resistance was mainly driven by the rpoB S531L (46.6%) mutation, followed by D516V (26.7%), H526Y (13.3%), and 6.6% for Q513L and P535L each. The predominance of rpoB Ser531Leu and Asp516Val mutations aligns with global reports identifying these as the most common rifampicin resistance–conferring mutations. Based on genotypic analysis of rifampicin resistance-determining region (RRDR) mutations across sub-Saharan Africa, S531L is consistently the most frequent mutation, followed by D516V and H526Y; as observed in Ethiopia (74.2%), in Angola (40%) , Angola (56%) ; and Nigeria [16–19]. These findings contrast somewhat with the earlier report from the Republic of Congo, where D516V was found more prevalent than S531L ; suggesting possible local transmission dynamics [16]. However, the present results may indicate a shift toward the regional pattern, which may reflect the selection pressure or temporal change in circulating strains [19].

About the catalase-peroxidase gene (KatG), the S315D mutation was the most prevalent, detecting around 66.7% of isolates. This finding is consistent with global data and aligns with regional mutations patterns reported in a comprehensive review encompassing 32 African countries [9,21]. The G279D mutation followed in frequency and is known to confer isoniazid resistance, although it is not yet included in the WHO mutation catalog or targeted by current Line Probe assay. This observation highlights the need for larger-scale studies to better characterize such uncommon mutations *thereby improving the accuracy of genotypic drug resistance profiling and guiding evidence-based treatment strategies* [22].

Among the *embB* gene mutations detected, Met306Val was the most frequent, representing a well-established marker of EMB resistance. This mutation alters the arabinosyl transferase enzyme involved in mycobacterial cell wall synthesis and reduces EMB binding affinity. The G406D mutation was observed in one isolate and has also been associated with EMB resistance, typically conferring low- to moderate-level resistance and often occurring alongside other mutations. Another single isolate carried the L355S substitution, not consistently listed in the WHO catalog but possibly acting as a compensatory or structural modification within the *embB* gene. These findings highlight the coexistence of both canonical and emerging embB mutations, underscoring ethambutol's continued importance in the intensive phase of tuberculosis treatment and the need for ongoing molecular surveillance to preserve its efficacy [23]

Regarding fluoroquinolone (FQ) resistance, three major gyrA mutations were identified: A90V (50%), D94G (33%), and S91P (17%). These frequencies align well with global data, where D94G typically accounts for ~30–50%, A90V for 15–40%, and S91P for 5–15% of FQ-resistant *M. tuberculosis* isolates [24–26]. The relatively high proportion of these mutations in the current dataset, despite a limited sample size, reinforces the need for broader FQ resistance surveillance, given their critical role in defining extensively drug-resistant tuberculosis (XDR-TB). These gyrA mutations are well recognized as primary mechanisms of resistance to second-line drugs such as ofloxacin, levofloxacin, and moxifloxacin, both in *M. tuberculosis* and in various Gram-negative bacteria

Among the rrs gene mutations, A1401G was detected in two of three isolates (67%), representing a well-characterized mutation conferring high-level resistance to aminoglycosides such as kanamycin and amikacin by altering the 16S rRNA target site, thereby reducing drug binding affinity. The G1484T mutation was identified in one isolate (33%), producing a similar structural modification that confers high-level resistance and may lead to cross-resistance among injectable second-line drugs. Collectively, these findings demonstrate the presence of key rrs mutations affecting the ribosomal

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binding site, emphasizing the importance of molecular resistance monitoring to guide effective therapy in MDR/XDR-TB cases [27].

We identified 13.6% rate of MDR-TB among the resistant isolates. This translates to an MDR-TB rate of 6.7% (3/45) in our overall cohort, which far exceeds the global average of around 3.5% for new TB cases. The findings mandates the need for the routine use of WHO-recommended rapid diagnostics (WRD) for all TB patients in our setting to ensure early detection and appropriate MDR-TB treatment initiation.

Demographic factors such as age, sex, and HIV status showed trends toward association with resistance but did not reach statistical significance, likely reflecting the limited sample size. Nonetheless, the observation of higher proportions of resistance among older individuals, females, and HIV-positive patients suggests potential epidemiological patterns that warrant further investigation in larger cohorts.

All mutations conferring resistance to first- and second-line anti-tuberculosis drugs were detected exclusively among Mycobacterium tuberculosis isolates, with no resistance-associated mutations identified in *M. africanum*. Although the limited sample size warrants cautious interpretation, this species-specific pattern aligns with a well-documented global trend. Extensive resources, including the WHO Catalogue of Mycobacterium tuberculosis Complex Mutations Associated with Drug Resistance and large-scale genomic surveillance studies, consistently demonstrate that the emergence, characterization, and transmission of drug-resistance determinants are overwhelmingly attributable to M. tuberculosis. Accordingly, this pathogen represents the principal driver of drug-resistant tuberculosis epidemics, whereas the rarity of resistance in other MTBC species likely reflects their comparatively lower transmission fitness, reduced exposure to selective drug pressure, and systematic underrepresentation in routine surveillance programs (15,28,29).

We acknowledge that the study is limited by a relatively small sample size of 45 *Mycobacterium tuberculosis* isolates. While this sample may not allow broad generalizations about drug-resistance prevalence in the region, it provides valuable molecular insights into the mutation profiles of first- and second-line anti-TB drugs in Congolese patients. Importantly, this study represents one of the first applications of MinION next-generation sequencing to characterize drug-resistance genes in this setting, enabling accurate detection of both common and rare mutations. Therefore, despite the limited number of isolates, the findings offer novel preliminary data that can inform larger epidemiological studies and guide regional TB management strategies.

5. Conclusions

This study provides an updated molecular snapshot of drug resistance in *Mycobacterium tuberculosis* isolates from the Republic of Congo. Predominant mutations rpoB S531L, KatG S315D, and embB Met306Val confirm globally recognized resistance markers for rifampicin, isoniazid, and ethambutol, while less common variants such as KatG G279D and embB Leu355Ser suggest ongoing evolution and potential regional specificity. The notable frequency of fluoroquinolone-associated gyrA mutations (A90V, D94G, S91P) and rrs mutations (A1401G, G1484T) highlights the risk of pre-XDR and XDR-TB. These findings underscore the need for comprehensive molecular surveillance, expanded sampling, and whole-genome sequencing to improve detection, guide treatment, and strengthen TB control in the region.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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Informed Consent Statement: Written informed consent was obtained from all adult participants. For minors, informed consent was obtained from their legal guardians, along with the child's assent when applicable.

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