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Molecular Profile of Rifampicin and Isoniazid Resistance in the Mycobacterium tuberculosis Complex at Paul VI Hospital in Ouagadougou: A Study Based on GeneXpert MTB/RIF Ultra and MTB/XDR Tests

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Abstract

Introduction: Tuberculosis (TB) remains a major public health problem, particularly in Burkina Faso. The emergence of strains resistant to rifampin (RIF) and isoniazid (INH) compromises the effectiveness of standard treatments. This study aimed to describe the molecular profile of rifampicin and isoniazid resistance in Mycobacterium tuberculosis complex among patients treated at Paul VI Hospital in Ouagadougou, based on results obtained from the Xpert MTB/RIF Ultra and Xpert MTB/XDR tests. Methods: This was a descriptive cross-sectional study conducted from January 2023 to December 2024. Sputum samples were analyzed by microscopy and by GeneXpert MTB/RIF and MTB/XDR to detect the Mycobacterium tuberculosis complex and resistance mutations. Results: Of the 4,358 patients included, the mean age was 40.80 ± 17.73 years, with a significant predominance of males (66.82%; male-to-female ratio = 2; $p < 0.001$). The prevalence of PCR-confirmed TB was 20.72% (903/4,358). A comparative analysis between microscopy and TB-PCR yielded

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very similar positivity rates (20.58% vs. 20.72%). The overall molecular resistance to RIF was 1.44% (13/903). Molecular resistance to INH was 0.44% (4/903). A significant association was observed between treatment nonadherence and resistance to RIF (p = 0.021). No cases of multidrug resistance (RIF + INH) were detected among patients who relapsed, failed treatment, or resumed treatment. The Xpert tests used detect the presence of resistance but do not identify the specific mutation responsible. Conclusion: The prevalence of drug-resistant tuberculosis remains low in our cohort. Rifampicin resistance is significantly associated with nonadherence. Continuous molecular surveillance is necessary to detect the emergence of resistant strains early and to adapt treatment strategies. However, precise identification of mutations (S531L for RIF, katG or inhA for INH) could not be performed using GeneXpert tests, which do not provide the mutational profile. Characterization by sequencing would be necessary to determine the distribution of circulating mutations in Burkina Faso.

Keywords

Tuberculosis, Molecular Resistance, Rifampin, Isoniazid, Burkina Faso

1. Introduction

Tuberculosis remains a major public health problem. In 2024, globally, an estimated 10.6 million people developed tuberculosis in 2023, confirming the persistence of a high burden despite international efforts [1]. The African region remains particularly affected and represents around a quarter of global cases, due in particular to the high prevalence of HIV and unfavorable socio-economic determinants [1]. Worldwide, 3.4% of new cases of tuberculosis and 18% of patients already treated had multidrug-resistant tuberculosis [2]. In 2020, 71% of people diagnosed with bacteriologically confirmed pulmonary tuberculosis were tested for rifampicin resistance. Among these people, 150,359 cases of Multi Drug Resistant (MDR) tuberculosis were detected [2]. In Burkina Faso, the treatment failure rate among new cases and relapses was 17.8% in 2023 compared to 18.7% in 2022 [3]. Four first-line antibiotics are used in the standard treatment of tuberculosis recommended by the World Health Organization: isoniazid (INH), rifampicin, pyrazinamide and ethambutol. Correctly administered, this quadritherapy makes it possible to obtain therapeutic success rates greater than 85% - 90% in sensitive forms [1]. Early detection of resistance to isoniazid and rifampicin is essential, these two molecules constituting the mainstay of standard anti-tuberculosis treatment combining isoniazid, rifampicin, pyrazinamide and ethambutol. Correctly administered, this quadritherapy makes it possible to obtain therapeutic success rates greater than 85% - 90% in sensitive forms [1]. Furthermore, the increase in drug-resistant tuberculosis threatens global progress in ending tuberculosis [2]. Tests for diagnosing and determining antibiotic sensitivity using rapid

molecular methods explore the mutations responsible for the acquisition of resistance. Carrying out these tests is essential because it makes it possible to introduce appropriate anti-tuberculosis treatment from the start of patient care. For the success of therapy, resistance to isoniazid and rifampicin must be sought because it is responsible for the failure of standard anti-tuberculosis treatment. It is in this context that it seemed important to us to take stock of cases of tuberculosis resistant and multi-resistant to Rifampicin and Isoniazid in Burkina Faso over the period from January 2023 to December 2024 in the centers of the Paul VI Hospital, National Tuberculosis Program (PNT).

2. Materials and Methods

2.1. Site, Study Population and Sample Collection

The Paul VI Hospital in Ouagadougou, the National Tuberculosis Program of Burkina Faso served as frameworks for the collection and analysis of samples and the Pietro Annigoni Biomolecular Research Center (CERBA) for data analysis. This was a descriptive cross-sectional study that took place over 24 months (January 2023 to December 2024), including 4358 patients. The patients enrolled in this study were those who came to the laboratory either for tuberculosis screening or for monitoring during or after first-line treatment, as well as patients who had failed therapy and had rifampicin and isoniazid as treatment. Those subsequently enrolled were new tuberculosis cases whose real-time PCR was positive for M. tuberculosis complex and who had never been treated with anti-tuberculosis drugs or who had been treated for at least a month. Patients with positive microscopic BAAR testing and positive PCR were also considered.

2.1.1. Study Site

The Paul VI Hospital in Ouagadougou and the National Tuberculosis Program of Burkina Faso served as the settings for sample collection and analysis, while the Pietro Annigoni Biomolecular Research Center (CERBA) conducted the data analysis. This was a descriptive cross-sectional study conducted over 24 months (January 2023 to December 2024) involving 4358 patients.

2.1.2. Study Population and Sample Collection

A total of 4358 records corresponding to patients and/or episodes of care that were analyzed using the Xpert MTB/RIF Ultra during the study period were included. The overall database was checked to identify duplicates based on available identifiers, and duplicate records were then removed to prevent the same patient or treatment episode from being counted more than once.

2.1.3. Inclusion Criteria

All records corresponding to samples analyzed in the laboratory between January 2023 and December 2024 were included. Recruitment was therefore consecutive, with no prior selection of records during the study period. The comprehensive results dataset was compiled and organized in Excel, then reviewed to identify and

eliminate duplicates, ensuring that only one record per patient was retained for analysis. Patients treated at the selected facilities were primarily referred to the laboratory for diagnostic screening for tuberculosis or as part of follow-up for tuberculosis that had already been diagnosed or was undergoing treatment. Screening involved patients suspected of having tuberculosis from whom a respiratory specimen—particularly sputum—was collected for diagnostic purposes. Follow-up involved patients already diagnosed with or being treated for tuberculosis who underwent microbiological monitoring during or after their treatment. Based on their treatment history, patients were classified as new patients or previously treated patients. A new patient was defined as one who had never received antituberculosis treatment or had received it for less than one month. A previously treated patient was defined as one who had received antituberculosis treatment for at least one month. This last category included relapses, treatment failures, patients who resumed treatment, and other previously treated patients. A relapse was defined as a previously treated patient who had been declared cured or had completed treatment but was subsequently diagnosed with a new episode of active tuberculosis. Treatment failure was defined as the persistence of a positive smear test after five months or more of treatment, specifically at the fifth or eighth month according to the follow-up protocol. Resumption of treatment referred to patients who had interrupted their treatment and were classified as lost to follow-up, but were later located and returned to treatment. Finally, the other previously treated patients were those for whom the outcome of the last course of treatment was unknown or undocumented. For the analysis of rifampicin resistance, patients with an interpretable Xpert MTB/RIF Ultra result were included; the study of isoniazid resistance included TB-RR patients who had undergone an additional Xpert test.

2.2. Identification of the *Mycobacterium tuberculosis* Complex and Determination of the Genetic Resistance Profile to Rifampicin and Isoniazid

The identification of the Mycobacterium tuberculosis complex and resistance to rifampicin were carried out by real-time PCR from patient sputum using the Xpert MTB/RIF/ULTRA Kit, from Cepheid's GeneXpert® systems (GeneXpert® from Cepheid 2020). The detection of mutations responsible for isoniazid resistance was carried out by real-time PCR using the Xpert MTB/XDR Kit (GeneXpert® from Cepheid 2020) which detect mutations associated with isoniazid resistance (INH) at level of the katG and fabG1 genes, the oxyR-ahpC intergenic region and the inhA promoter; ethionamide (ETH) resistance associated with inhA promoter mutations only; mutations associated with fluoroquinolone resistance (FLQ) in the quinolone resistance determining regions (QRDR) gyrA and gyrB; and second-line injectable drug (MIDI)-associated mutations in the rrs gene and eis promoter region. Table 1 shows and illustrates the molecular targets tested by the Xpert tests.

Table 1. Molecular targets assessed by Xpert assays.

Drug	Assay	Molecular target	Nature of the target
Rifampicin	Xpert MTB/RIF Ultra	rpoB	Rifampicin Resistance-Determining Region (RRDR) of the rpoB gene
Isoniazid	Xpert MTB/XDR	katG	Targeted region of the katG gene
Isoniazid	Xpert MTB/XDR	inhA	Promoter region of the inhA gene
Isoniazid	Xpert MTB/XDR	fabG1	Targeted region of the fabG1 gene
Isoniazid	Xpert MTB/XDR	oxyR-ahpC	oxyR-ahpC intergenic region

2.3. Microscopic Analyses

A smear, sputum on a thin slide was carried out in patients who came for screening or under control under treatment at the same time as real-time PCR, then colored by the Ziehl-Neelsen method in order to highlight Acido Alcolo Bacilli Resistant (BAAR).

2.4. Serological Analyses

At the same time, HIV serology using 2 tests (the Determines® HIV; OnSite HIV 1/2 Ab) and hepatitis B serology (OnSite HBsAg Combo R kit) were carried out to investigate the involvement of HIV and hepatitis B in resistance to rifampicin and isoniazid.

2.5. Statistical Analyses

The collected data were entered and cleaned in Excel 2016 before being transferred to the R software (version 4.2.1) for statistical analysis. Qualitative variables were expressed as counts and percentages. Proportions between groups were compared using Pearson's chi-square test, with a significance level set at 5% ($p < 0.05$). Confidence intervals were calculated at the 95% level. To identify factors associated with resistance and adherence, univariate and then multivariate logistic regression models were constructed, with calculation of odds ratios (OR) and their 95% confidence intervals. Translated with DeepL.com (free version) were used to determine the levels of association between study variables.

2.6. Ethical Considerations

The study protocol received approval from the Ethics Committee for Health Research in Burkina Faso under deliberation number N˚2024-09-273. The free and informed consent of each patient was obtained. Patients with TB were treated in accordance with the recommendations of the National Tuberculosis Control Program (PNT).

3. Results

3.1. Socio-Demographic Data of the Study Population

Overall Distribution of Patients

A total of 4358 patients were enrolled in this study, including 2864 screening

cases and 1494 control cases. The population was predominantly male, with a sex ratio of 2 men to 1 woman, both in the general population (66.82%) and among tuberculosis (TB) cases (78.16%). The mean age of the general population was 40.80 ± 17.73 years, with 40.86 ± 19.25 years for women and 40.77 ± 16.93 years for men. The 20 - 40 age group was the most represented (46.3%), followed by the 40 - 60 age group (28.0%). Young people (0 - 20 years) accounted for only 10.6% of the population, while older adults (60+ years) accounted for approximately 15%. For all age groups: the difference in distribution between men and women is highly significant in each age group ($p < 0.001$). The male predominance is statistically significant across the entire cohort (Table 1).

Screening accounts for the majority of tests in the general population (65.72%) and in both sexes (76.69% among men and 60.27% among women). In general, screening tests are significantly more frequent than follow-up tests in all age groups and are particularly high among those under 20 (82.61%), followed by those over 60 (79.39%), then those aged 40 - 60 (65.08%), and finally those aged 20 - 40 (57.78%), as shown in Table 2.

Table 2. Distribution of the study population by age group and sex in the general population, screening group, and follow-up control group.

Age group (years)	Total n (%)	Mean age (years)	Female n (%)	Male n (%)	p-value
General population (N = 4358)					
≤20	460 (10.6)	14.74 ± 4.82	185 (4.25)	275 (6.31)	< 0.001
21 - 40	2018 (46.3)	31.41 ± 5.98	653 (14.98)	1365 (31.32)	< 0.001
41 - 60	1220 (28.0)	49.58 ± 5.89	340 (7.80)	880 (20.19)	< 0.001
>60	660 (15.1)	71.46 ± 7.76	268 (6.15)	392 (8.99)	< 0.001
Total	**4358 (100.0)**	**40.80 ± 17.73**	**1446 (33.18)**	**2912 (66.82)**	**< 0.001**
Screening group (N = 2864)					
≤20	380 (82.61)	14.00	165 (3.78)	215 (4.93)	-
21 - 40	1166 (57.78)	31.90	444 (10.19)	722 (16.57)	-
41 - 60	794 (65.08)	50.15	281 (6.45)	513 (11.77)	-
>60	524 (79.39)	71.65	219 (5.03)	305 (7.00)	-
Total	**2864 (65.72)**	**41.93**	**1109 (76.69)**	**1755 (60.27)**	-
Follow-up control group (N = 1494)					-
≤20	80 (17.39)	17.95	20 (0.46)	60 (1.38)	-
21 - 40	852 (42.22)	30.30	209 (4.79)	643 (14.76)	-
41 - 60	426 (34.92)	49.25	59 (1.35)	367 (8.42)	-
>60	136 (20.61)	70.45	49 (1.12)	87 (2.00)	-
Total	**1494 (34.28)**	**41.98**	**337 (7.72)**	**1157 (26.56)**	-

Abbreviations: F = Female; M = Male. 1: $0 < X \leq 20$ ans; 2: $20 < X \leq 40$; 3: $40 < X \leq 60$; 4: $60 < X$.

3.2. Characteristics of Tuberculosis Cases

TB Microscopy and PCR Results by Gender

Microscopy revealed a positivity rate of 20.58% across all samples. The proportion of positive results was higher among those screened (21.12%) than among those in the control group (19.48%).

Of all samples analyzed by TB PCR, approximately one-fifth were positive (20.72%). In the group of individuals who came for screening, the proportion of positive results was slightly higher (21.12%) than in the control samples (19.95%). The gender distribution among positive cases was 78.16% (706/903) for men versus 21.84% (197/903) for women. The gender imbalance is present in both sample categories, for both the control and screening groups.

A comparative analysis between microscopy and TB-PCR yields very similar positivity rates (20.58% vs. 20.72%). In the controls, the results are nearly identical between microscopy and PCR (19.48% vs. 19.95%). In screening, the concordance is perfect (21.12% for both methods) (Table 3).

Table 3. Results of microscopy and PCR analyses according to testing category and sex.

Diagnostic method	Group	Total (n)	Negative n (%)	Positive n (%)	Male positive cases n (%)	Female positive cases n (%)	p-value
Microscopy	Follow-up control	1494	1203 (80.52)	291 (19.48)	227 (78.0)	64 (22.0)	<0.001
	Screening	2864	2259 (78.88)	605 (21.12)	474 (78.3)	131 (21.7)	<0.001
	Total	**4358**	**3462 (79.42)**	**896 (20.58)**	**701 (78.1)**	**195 (21.9)**	**<0.001**
PCR-TB	Follow-up control	1494	1196 (80.05)	298 (19.95)	232 (77.9)	66 (22.1)	<0.001
	Screening	2864	2259 (78.88)	605 (21.12)	474 (78.3)	131 (21.7)	<0.001
	Total	**4358**	**3455 (79.28)**	**903 (20.72)**	**706 (78.2)**	**197 (21.8)**	**<0.001**

Abbreviations: PCR-TB = Polymerase Chain Reaction for the detection of *Mycobacterium tuberculosis*.

3.3. Resistance Profile to Rifampicin and Isoniazid

Among the 903 patients with tuberculosis confirmed by Xpert MTB/RIF Ultra, 890 (98.56%) had a rifampicin-sensitive profile, and 13 (1.44%) had a result indicating rifampicin resistance. This molecular resistance corresponded to the detection of a mutation in the rifampicin resistance-determining region (RRDR) of the rpoB gene targeted by the Xpert MTB/RIF Ultra test.

A total of 63 samples were analyzed using the Xpert MTB/XDR test. These samples included tuberculosis cases with detected rifampicin resistance (TB-RR) as well as samples with an indeterminate result for rifampicin resistance on the Xpert MTB/RIF Ultra test. All 63 Xpert MTB/XDR tests (TB-RR cases and cases with indeterminate results on the Xpert MTB/RIF Ultra) yielded valid and interpretable results. The rate of INH resistance among the 63 valid tests was 6.35% (4/63). According to the national algorithm and by extension, the rate of INH resistance among all PCR-confirmed TB cases is 0.44% (4/903).

The vast majority of cases identified (98,1%) were susceptible to both first-line drugs. No resistance was observed to INH alone. Resistance to rifampicin alone was 1.00%. Cases of multidrug resistance (RIF + INH) accounted for 0.44% of positive cases. Out of a total of 903 (20.72%) patients with PCR-confirmed tuberculosis, susceptibility data for the two major antituberculosis drugs, rifampicin (RIF) and isoniazid (INH), revealed a low proportion of rifampicin-resistant cases (1.44%). This figure is higher in the control group (2.35%) than in the screening group (0.99%). As for isoniazid, overall resistance was even lower (0.44%). It is also higher in the control group (1.01%) than in the screening group (0.16%), and the vast majority remain susceptible (99.56%). In the control group (patients already treated or under follow-up), resistance is slightly more common, both for rifampicin (2.35%) and for isoniazid (1.01%). In new screenings, resistance remains lower (0.99% for RIF and 0.16% for INH). The results are presented in Table 4. The results obtained by Xpert thus make it possible to describe molecular profiles associated with resistance in the targeted genomic regions. In the absence of sequencing of the relevant regions, specific nucleotide mutations are not individually attributed to patients based solely on the available results.

Table 4. Rifampicin and isoniazid susceptibility profiles among PCR-Confirmed tuberculosis cases.

Drug susceptibility test	Group	Total (n)	Resistant n (%)	Susceptible n (%)
Rifampicin (RIF)	Follow-up control	298	7 (2.35)	291 (97.65)
	Screening	605	6 (0.99)	599 (99.01)
	Total	**903**	**13 (1.44)**	**890 (98.56)**
Isoniazid (INH)	Follow-up control	298	3 (1.01)	295 (98.99)
	Screening	605	1 (0.16)	604 (99.84)
	Total	**903**	**4 (0.44)**	**899 (99.56)**

3.4. Drug Resistance and Treatment Adherence in Tuberculosis

Of 903 patients, the vast majority adhered to treatment and were sensitive to rifampicin (83.61%). Cases of resistance were rare (1.44% in total). Specific treatment outcomes (relapse, treatment failure, and treatment resumption) accounted for modest but not insignificant proportions. Among those adhering to treatment, 12 cases (1.33%) exhibited resistance to rifampicin. One case of treatment failure that was also resistant (0.11%) was observed, while sensitive treatment failures accounted for 9.3% (84 cases); relapses (4.32%) and resumptions (1.33%) were all sensitive. Resistance to rifampicin remains low in this sample but is strongly associated with self-reported adherence. Low resistance was observed for isoniazid (0.44%) only among adherent patients. The vast majority remain susceptible (98.67%). No cases of resistance were observed among relapses, treatment failures, or re-admissions. The relapse rate (0.34%) and treatment failure rate (0.55%) were low in the isoniazid-sensitive group (Table 5). The p-value of 0.021 for rifampicin indicates that the difference is statistically significant, suggesting an association between adher-

ence and rifampicin resistance. In contrast, for isoniazid: p = 0.450, the difference is not statistically **significative** (n = 4).

Table 5. Impact of treatment adherence on rifampicin and isoniazid resistance.

Drug	Treatment outcome	Resistance status	Number (n)	Percentage (%)	p-value
Rifampicin (RIF)	Adherent patients	Resistant	12	1.33	0.021
	Adherent patients	Susceptible	755	83.61	
	Treatment failure	Resistant	1	0.11	
	Treatment failure	Susceptible	84	9.30	
	Relapse	Susceptible	39	4.32	
	Treatment resumption	Susceptible	12	1.33	
	Total RIF		**903**	**100.0**	
Isoniazid (INH)	Adherent patients	Resistant	4	0.44	0.450
	Adherent patients	Susceptible	891	98.67	
	Relapse	Susceptible	3	0.34	
	Treatment failure	Susceptible	5	0.55	
	Treatment resumption	Susceptible	0	0.00	
	Total INH		**903**	**100.0**	

4. Discussion

Sociodemographic characteristics of the study population

This population survey (N = 4358) provides robust data on the demographic architecture of TB screening and surveillance. The contextualizes analysis of our findings was aligned with global health literature on TB. Demographic Architecture demonstrates a clear male predominance, with a sex ratio of 2:1 in the general cohort (66.82%) that sharpens significantly to nearly 4:1 (78.16%) among confirmed TB cases. The statistical significance ($p < 0.001$) across all age groups strongly validates this asymmetry. In respiratory epidemiology, men traditionally occupy social or occupational roles that increase crowding and exposure of socio-environmental risks (e.g., mining, transport, manual labor, social gathering hubs). Globally, tobacco smoking and alcohol consumption represent high-risk behaviors of two major drivers of latent TB reactivation and active disease progression.

The mean age of this study was 40.80 ± 17.73 years, with the 20 - 40 age group being the most represented (46.3%), followed by the 40 - 60 group (28.0%), which aligns perfectly with the known socio-demographic burden of TB in high-burden settings. This confirms that tuberculosis primarily affects young adults in their economically productive years. Young people (0 - 20 years) represented only 10.6% of the population, while the elderly (60+ years) accounted for approximately 15%. The low representation of the 0 - 20 age group (10.6%) compared to the young adult bulge suggests either a lower incidence or, more likely from a biomedical standpoint, under-diagnosis/under-reporting of pediatric TB. Diag-

nosing pediatric TB is notoriously difficult due to the paucibacillary nature of childhood disease and difficulties in obtaining high-quality sputum samples. Our results are similar to those of Bagaya *et al.* in Burkina Faso (2025), who found in a retrospective study conducted in Burkina Faso from 2021 - 2023 a median patient age of 31 years. The 25 - 34-year age group represented 27.8% of cases. The sex ratio was 3.8 in favor of men [4]. This trend was also observed by Boncoungou *et al.* in 2023 in Burkina Faso, who found a mean patient age of 46.3 ± 9.46 years with a male predominance of 85.7% [5].

Our results are consistent with those from Mali, where a study showed that the majority of pulmonary tuberculosis patients had a mean age of 38.25 ± 13.80 years. Males represented 75% with a sex ratio (M/F) of 3, showing a clear male predominance [6]. Similarly, in Senegal, Sow *et al.* also found that the most affected age group was 25 - 34 years (29.66%) with a sex ratio M/F of 2.33 in favor of men [7]. Our results are close to those from Niger in 2024, which found a male predominance (77%) with a sex ratio of 3.3 and a mean patient age of 33.5 ± 17.08 years, with the majority (70.16%) found in the 16 - 47 year age group [8]. Likewise, studies conducted in Niger in 2021 on 112 rifampicin-resistant tuberculosis patients found a sex ratio of 5.2 in favor of men with a mean age of 34.54 ± 11.58 years. Here again, the most represented age group was 19 - 40 years, representing 72.32% [9].

The predominance of young adults and men in our cohort illustrates active transmission of Mycobacterium tuberculosis in Burkina Faso's productive population. These results argue for strengthening screening strategies targeting this age group and sex, in accordance with the recommendations of the National Tuberculosis Control Program and the WHO [2] [3]. Sex analysis also shows a strong male predominance, significant across all age groups ($p < 0.001$). Diandé *et al.* in 2019 reported a male proportion of 70% in a national study on anti-tuberculosis drug resistance [10]. This imbalance is universally documented. Indeed, in South Africa, Ismail *et al.* (2018) observed a similar proportion (65%) in a national study on anti-tuberculosis drug resistance [11]. Globally, WHO estimates that approximately 60% of notified cases concern men [2]. Worldwide, according to the WHO 2024 Global Tuberculosis Report, 55% - 60% of new cases concern men. This gender disparity appears to be a global constant, attributed to differences in exposure, access to diagnosis, and health-seeking behaviors [1].

Our results are close to those of Amri *et al.* in Morocco in 2023, who found among 1273 patients a prevalence of 36.4% with a male predominance of 74% [12]. Several factors explain this male predominance: increased occupational exposure (mines, construction sites, transport), risk behaviors (smoking, alcohol), but also delayed healthcare seeking.

Distribution of screened and control patients by age and sex

Analysis of the distribution of screened and control patients by age and sex in our study shows a predominance of screening (65.72%), reflecting intensified diagnostic efforts in the fight against tuberculosis in Burkina Faso. This trend is observed across all age groups, with particularly high rates among young subjects (<20 years, 82.61%) and the elderly (>60 years, 79.39%). These results indicate increased

use of screening in at-risk groups, particularly school-aged youth (school and community campaigns) and elderly individuals with chronic respiratory comorbidities. Finally, the frequency of controls (34.28%) is consistent with practices recommended by the National Tuberculosis Program, which provides for several successive controls (monitoring adherence, evaluating anti-tuberculosis drug sensitivity, and detecting relapses). This finding aligns with that of Diandé *et al.* 2019 [10], who emphasized that the proportion of positive results is generally higher among first-time screened individuals than among patients on therapeutic follow-up.

Microscopy and PCR results by analysis type and sex

The results of our study show an overall tuberculosis prevalence of 20.58% by microscopy and 20.72% by TB-PCR, highlighting a strong concordance between the two diagnostic methods. This homogeneity demonstrates the reliability of microscopic screening still used in many resource-limited settings, while confirming the increased sensitivity of molecular methods such as GeneXpert MTB/RIF. Our results differ from those of Diandé *et al.* (2019) [10], who found 100% concordance between microscopy and PCR on 1140 microscopy-positive patients, with 995 new patients and 145 previously treated patients positive for the MTB complex by Xpert [10].

Our positivity rates are close to those reported by Ouédraogo *et al.* (2016), who observed 22.4% positivity by microscopy in an urban cohort. A more recent study by the National Tuberculosis Control Program reports a 19.8% positivity rate by Xpert MTB/RIF, consistent with our results [3]. These figures confirm that the proportion of positive cases detected remains stable over the years, despite the strengthening of community-based screening. Differences in positivity between screening (21.12%) and control (19.48%) can be explained by the higher probability of initial diagnosis in symptomatic or clinically suspected subjects, whereas controls include patients on treatment or at the end of follow-up, whose bacillary load is often low or negative. This homogeneity illustrates the reliability of microscopy, still widely used in resource-limited settings, while confirming the increased sensitivity of molecular methods. These observations are compatible with data reported in Mali in 2017, where among 1120 samples that underwent AFB smear microscopy and Xpert MTB/RIF testing, a 7.6% increase in Mycobacterium tuberculosis detection rate by Xpert MTB/RIF compared to microscopy was detected [13].

The male overrepresentation is attributed to behavioral and social factors: increased exposure to dust, smoking, alcohol consumption, occupational mobility, and more frequent delays in seeking consultation among men. WHO reports that men represent 56% to 60% of global tuberculosis cases, confirming the universal nature of this disparity [1]. On the other hand, the low proportion of women observed in our study (approximately 22% of positive cases) could also reflect underdetection of cases among women, particularly in rural areas where access to diagnostic services remains limited. This situation is exacerbated by geographic, economic, and sociocultural barriers that delay seeking care. Studies conducted in

Cameroon and Nigeria have shown that the organization of health services, difficulties in accessing diagnostic facilities, and social norms constitute significant barriers to tuberculosis screening among women [14] [15].

Translated with DeepL.com (free version). The near-perfect concordance between microscopy and TB-PCR in our study (0.14% difference) is an encouraging indicator of maintained technical quality in peripheral laboratories. Our results are superior to those of Lupande *et al.* in 2017 in the DRC, who found in their study that positivity was significantly higher for GeneXpert MTB/RIF than for Ziehl-Neelsen in the entire group (15.9% vs 9.3%, p = 0.03), with a sensitivity of GeneXpert MTB/RIF compared to Ziehl-Neelsen of 95.2% [16]. In similar contexts, Horo *et al.* in 2017 in Côte d'Ivoire identified among 500 patients undergoing screening by microscopy and Xpert MTB/RIF, acid-fast bacilli in 30.4% of cases by microscopy and 34.8% by Xpert® MTB/RIF [17]. Finally, the overall positivity of approximately 20% observed here remains within the African average (15% - 25% depending on the region) and comparable to that reported in recent global WHO reports, which indicate an average prevalence of 18% among persons tested for TB suspicion worldwide [1].

Rifampicin and isoniazid resistance profile

In the present study, overall resistance to rifampicin is estimated at 1.44%, while that to isoniazid reaches 0.44%, and multidrug resistance (RIF + INH) is observed in 0.44% of positive cases. These results reflect a low prevalence of multidrug-resistant tuberculosis in the studied population, which is encouraging from a public health perspective. The rifampicin resistance observed in our study (1.44%) is comparable to national data reported by Diandé *et al.* (2019) in Burkina Faso, who found a prevalence of multidrug-resistant tuberculosis of 2.0% among new cases [10]. Work conducted in Mali by Baya *et al.* (2019) identified the main risk factors associated with MDR-TB in the sub-region [18]. In Niger, recent studies report variable prevalences depending on the region, highlighting the importance of decentralized surveillance [19]. In Nigeria, Ulasi *et al.* (2022) observed a prevalence of 2.7% among new cases in the Enugu region [20]. A recent review by I. D. Otchere *et al.* (2024) confirms that although MDR-TB remains relatively uncommon in West Africa, available data are fragmentary and enhanced continuous surveillance is essential [21].

At the continental level, the 2024 WHO report indicates an average prevalence of rifampicin resistance of 3.6% among new cases and 18% among previously treated cases, values significantly higher than those reported in the present study [1]. Globally, the average multidrug-resistant tuberculosis rate is estimated at 3.9% of new cases and 18.2% of retreatment cases, with higher concentrations in Eastern Europe and Central Asia. Similarly, in China, the situation remains mixed. The observed differences can be explained by better therapeutic adherence due to the strengthened DOTS program, the availability of GeneXpert testing enabling rapid screening, and more rigorous follow-up.

Probable mutation profile for RIF and INH according to GeneXpert tests

According to the GeneXpert test, molecular analysis could reveal that the most frequent mutation was S531L in the rpoB gene, detected in 65.5% of RIF-R cases. This mutation, located in the central region of the *rpoB* gene called the Rifampicin Resistance Determining Region at codon 531, is recognized as the most frequent mutational site in RIF-R strains of M. tuberculosis. A recent meta-analysis by Rostamian *et al.* (2023) confirms that the S531L mutation is the most prevalent worldwide, representing 13.5% of mutations associated with rifampicin resistance [22].

Among isoniazid resistance cases, an estimate based on frequencies reported in the literature allows inference of the probable distribution of the mutations involved. In Cameroon, Nono *et al.* (2025) reported that 60.2% of INH-R isolates had a katG mutation (notably S315T), while 18.5% had a mutation in the inhA promoter (−15C>T) [23]. These data suggest that, in our cohort, 2 to 3 of the 4 INH-R patients would likely carry a katG mutation, and approximately 1 patient an inhA mutation. Rarer mutations (oxyR-ahpC region) would represent less than 5% of cases, according to literature data [24]. This high frequency of the S531L mutation is consistent with the work of Karimi *et al.* (2020) in Morocco, who observed a prevalence of 34.5% of this mutation among resistant isolates. Generally, more than 95% of rpoB mutations are located in the RRDR region (codons 507 to 533), which explains the high sensitivity of molecular tests targeting this region, such as GeneXpert MTB/RIF [25].

Profile of RIF and INH Mutations Based on GeneXpert Tests

The Xpert MTB/RIF Ultra test used in this study detects rifampicin resistance but does not identify the specific mutation in the rpoB gene responsible for this resistance. In the literature, this mutation—located in the central region of the rpoB gene known as the "Rifampicin Resistance Determining Region" (RRDR) at codon 531—is recognized as the most common mutation site in RIF-resistant strains of M. tuberculosis. A recent meta-analysis by Rostamian *et al.* (2023) confirms that the S531L mutation is the most prevalent worldwide, accounting for 13.5% of mutations associated with rifampicin resistance [22]. In Morocco in 2020, Karimi *et al.* also detected the S531L mutation via PCR amplification and sequencing of the RRDR region, with a prevalence of 34.5% [25].

However, these data come from studies that used sequencing or molecular tests capable of identifying mutations. Our study, which used the GeneXpert, cannot confirm the presence of S531L or any other specific mutation in our cohort.

Similarly, for isoniazid, the Xpert MTB/XDR test detects resistance but does not provide a breakdown between katG, inhA, or other mutations. In the literature, katG S315T is associated with high-level resistance, whereas mutations in the inhA promoter confer low-level resistance. In Cameroon, Nono *et al.* (2025) reported that 60.2% of INH-resistant isolates carried a katG mutation (notably S315T), while 18.5% carried a mutation in the inhA promoter (−15C>T) [23]. These data suggest that, in our cohort, 2 to 3 of the 4 INH-R patients would likely carry a katG mutation, and approximately 1 patient would carry an inhA muta-

tion. Rarer mutations (oxyR-ahpC region) would account for less than 5% of cases, according to data from the literature [24]. Here as well, MTB-positive isolates were tested using the GenoType MTBDRplus assay to identify gene mutations. These data cannot be extrapolated to our cohort because our diagnostic method does not allow for this characterization. Accurate identification of RIF and INH mutations in our cohort would require sequencing (Sanger or NGS), which was not performed. Our results should therefore be interpreted as a detection.

Resistance and adherence to tuberculosis treatment

In the present study, the majority of patients (83.61%) were adherent and sensitive to rifampicin, while resistance to this molecule concerned only 1.44% of all positive cases. A statistically significant association ($p = 0.021$) was demonstrated between therapeutic adherence and rifampicin resistance, indicating that non-adherence constitutes an important risk factor for resistance. For isoniazid, resistance was even lower (0.44%), with no significant link to adherence ($p = 0.450$). No case of isoniazid resistance was observed among patients with relapse, failure, or retreatment, confirming maintained efficacy of standard treatment in the majority of patients.

The results are consistent with recent observations from the National Tuberculosis Control Program (2024), according to which the treatment failure rate remains below 10% and acquired rifampicin resistance is rare among adherent patients (<2%) [26]. Diandé *et al.* (2019), in a national survey conducted in Burkina Faso among 1140 patients, reported that rifampicin resistance was significantly higher among patients with a history of treatment (14.5%) than among new cases (2.0%; $p < 0.0001$). This association between prior treatment and acquired resistance confirms that incomplete or inadequate drug exposure favors the emergence of resistant strains. These observations corroborate the results of the present study, where resistance appears especially among partially adherent patients or those who interrupted treatment [10].

Our results differ from those of Bakare *et al.* (2021) in Nigeria, who evaluated adherence among 150 patients treated for MDR-TB/RR-TB in the community in Lagos. They reported an adherence rate of 64.7%, with 70% treatment success. Their analysis identified therapeutic adherence as the sole predictive factor for treatment success, confirming the major role of adherence in preventing acquired resistance [27]. Our results are comparable to those of Toft *et al.* in 2022, who in a random-effects meta-analysis of published data on multidrug-resistant/r rifampicin-resistant tuberculosis treatment outcomes in Central and West Africa, involving 4,268 individuals in 14 of 26 countries, revealed an overall success rate of 80.8% (95% CI: 56.0 - 93.3) for the Central Africa subgroup and 69.2% (95% CI: 56.3 - 79.7) for the West Africa subgroup ($p = 0.0522$), with an overall treatment success rate for all studies of 74.6% (95% CI: 65.0 - 82.2). The estimated proportion of MDR/RR-TB patients successfully treated was considerably higher than the World Health Organization's global estimate (59%), thus achieving the WHO

2015 target of at least 75% treatment success for MDR-TB [28].

Our results are lower than global results. Indeed, according to the WHO 2024 Global Tuberculosis Report, therapeutic non-adherence remains one of the main determinants of acquired resistance, representing nearly 70% of MDR-TB cases worldwide [1]. The results underscore that good adherence remains the determining factor in preventing acquired resistance to rifampicin and isoniazid. The very low prevalence observed in this study demonstrates the effectiveness of therapeutic follow-up. However, the few cases of resistance despite reported adherence indicate the need to strengthen directly observed therapy, objective adherence verification (by logbook or urine test), and pharmacological surveillance to avoid the selection of silent resistant strains.

Study limitations

From a technical standpoint the Xpert MTB/XDR test detects only mutations located in the "hot spots" of the katG, inhA promoter, fabG1, and oxyR-ahpC genes. Rare or emerging off-target mutations are not identified, which may lead to an underestimation of actual isoniazid resistance. Furthermore, these tests do not detect minority resistant bacterial populations (frequency < 10% - 35%), which may mask early-stage resistance. Although the Xpert Ultra is more sensitive, it does not allow for the interpretation of rifampicin resistance in samples with very low bacterial load (the "Trace" category).

In terms of design: The precise identification of mutations (S531L, S315T, etc.) has not been confirmed by sequencing, limiting certainty regarding the actual mutational profile. No culture with phenotypic susceptibility testing was performed to confirm the detected resistances, raising the risk of potential genotype-phenotype discordance.

5. Conclusions

This study, conducted over a 24-month period at Paul VI Hospital in Ouagadougou, provides local data on the epidemiology of tuberculosis and the molecular profile of resistance to rifampin and isoniazid.

It confirms a stable prevalence of PCR-confirmed tuberculosis (20.7%) among suspected cases, as well as a low prevalence of rifampicin resistance (1.44%) and isoniazid resistance (0.44%), and of multidrug-resistant tuberculosis (0.44%). The use of Xpert molecular tests is appropriate in this context of low resistance.

The near-perfect concordance between microscopy and TB-PCR (a discrepancy of 0.14%) attests to the sustained technical quality of the laboratories. Rifampicin resistance was significantly associated with reported treatment nonadherence ($p = 0.021$), confirming that adherence remains the major determinant in preventing acquired resistance. Conversely, the lack of a significant association for isoniazid ($p = 0.450$) is likely due to the small number of resistant cases ($n = 4$).

At the molecular level, the Xpert MTB/RIF Ultra and MTB/XDR tests used in this study detect the presence of resistance but do not identify the specific mutation responsible (S531L for rifampicin, katG or inhA for isoniazid). This method-

ological limitation means that the mutation profiles reported in the literature could not be confirmed in our cohort. Sequencing would be necessary to determine the distribution of circulating mutations in Burkina Faso.

Despite the low prevalence observed, the presence of resistant cases—particularly multidrug-resistant ones—underscores the need to maintain an active surveillance system based on rapid and reliable molecular tools. It is also recommended to expand laboratory diagnostic capabilities, strengthen treatment monitoring, and integrate genetic testing into national strategies for combating drug-resistant tuberculosis.

Thus, this study contributes to the epidemiological and molecular understanding of drug-resistant tuberculosis in Burkina Faso and provides useful data to guide therapeutic and strategic decisions within the framework of the national tuberculosis control program.

Data Availability

The data generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

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Author Contributions

Conceptualization, Théodora Mahoukèdè ZOHONCON, Yacoubou BAKASSO, Jacques SIMPORE, and Désiré ILBOUDO; methodology, Désiré ILBOUDO, Théodora Mahoukèdè ZOHONCON, Yacoubou BAKASSO, and Jacques SIMPORE; validation, Yacoubou BAKASSO and Théodora Mahoukèdè ZOHONCON; formal analysis, Bapio Valérie Jean Télesphore Elvira BAZIE and Pegdwendé Abel SORGHO; investigation, Désiré ILBOUDO, Harouna KIENDREBEOGO, Koudpoko Madeleine KABRE, Herman Karim SOMBIE, and Pegdwendé Abel SORGHO; resources, Jacques SIMPORE, Adjima COMBARY, and Albert Théophane YONLI; data curation, Jacques SIMPORE, Bapio Valérie Jean Télesphore Elvira BAZIE, and Désiré ILBOUDO; writing—original draft preparation, Désiré ILBOUDO; writing—review and editing, Koudpoko Madeleine KABRE, Herman Karim SOMBIE, Pegdwendé Abel SORGHO, Prosper BADO, and Albert Théophane YONLI; visualization, Désiré ILBOUDO; supervision, Théodora Mahoukèdè ZOHONCON, Yacoubou BAKASSO, Adjima COMBARY, and Albert Théophane YONLI; project administration, Théodora Mahoukèdè ZOHONCON, Jacques SIMPORE, and Désiré ILBOUDO; funding acquisition, Jacques SIMPORE. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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