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Molecular Detection of Multidrug-Resistant Tuberculosis Reveals High Prevalence among Previously Treated Tuberculosis Patients in Burkina Faso

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Abstract

Multidrug-resistant tuberculosis (MDR-TB) is a major public health problem worldwide, particularly in Eastern Europe, Russia, and Sub-Saharan Africa due to its prevalence, burden, treatment management difficulties, and socio-economic impacts. Burkina Faso is also facing the challenge of MDR-TB, which is a major public health threat in the country, given the trends in the prevalence and burden of the disease in recent years. The effective control of tuberculosis for its elimination needs to strengthen the prevention and control of MDR-TB. Therefore, assessment, efficient diagnostic tools, prevention of transmission, and effective management of treatment of MDR-TB are very important key measures. The aim of this study was to assess the prevalence of MDR-TB within new and previously treated tuberculosis cases in Burkina Faso. It is a descriptive cross-sectional study conducted from October 2022 to

March 2023 at the National Reference Laboratory for Mycobacteria, Ouagadougou, Burkina Faso. Molecular diagnostic tests such as Xpert MTB/RIF and GenoType MTBDR*Plus* v1, a line probe assay (LPA), were used to detect *Mycobacterium tuberculosis* complex and to identify drug resistance-associated gene mutations to predict resistance. Among 500 suspected tuberculosis patients' samples analyzed by the Xpert MTB/RIF assay, 169 (33.7%) patients were positive for *Mycobacterium tuberculosis* complex, including 100 (59.17%) new TB cases and 69 (40.83%) previously treated patients. Of these 169 TB-positive cases, the global prevalence of MDR-TB predicted based on Xpert MTB/RIF results was 24.26% (41/169). Considering new TB cases (n = 100) and previously treated TB cases (n = 69), the MDR-TB prevalence was very high in previously TB-treated cases, 40.58% (28/69) compared to new TB cases, 13% (13/100). Of the 41 rifampin-resistant cases (or MDR-TB cases based on prediction) detected by Xpert MTB/RIF, a subset of 35 samples (randomly selected) were analyzed by the MTBDR*Plus* V2 test and revealed 5. 71% (2/35) mono-resistant to rifampicin, 14.28% (5/35) mono-resistant to isoniazid and 74.29% (26/35) multi-resistant (rifampicin + isoniazid). The main genes associated-mutations conferring drug resistance to rifampicin and isoniazid determined by the GenoType MTBDR*Plus* V2 on the subset strains (N = 35) were D516V (20%), S531L (20%), H526D (15.29%) in *rpoB* gene for rifampicin resistance and S315T1 (80%), T8C (14.29%) in *katG* and *inhA* genes for isoniazid resistance. These mutations are the main ones described and targeted by the Nucleic Acid Amplification Tests (Xpert MTB/RIF, GenoType MTBDR*Plus* V2, and others). Therefore, Xpert/MTB-RIF and MTBDR*Plus* tests are expected to be performant in Burkina Faso for the detection of resistance to rifampicin or rifampicin plus isoniazid, as the conferring resistance mutations circulating in this geographical area are mainly those covered by these tests.

Keywords

Multidrug-Resistant Tuberculosis, Molecular Detection, Xpert MTB/RIF, GenoType MTBDR*Plus*, Resistance Genes Mutations, Burkina Faso

1. Introduction

Tuberculosis (TB) is an infectious, contagious, and curable disease, endemic and epidemic, with essentially human-to-human transmission [1]. TB continued to be a high global health concern as it is the second leading cause of infectious disease mortality with around 1.4 million deaths in 2021 [2]. Drug-resistant tuberculosis particularly multi-drug-resistant TB (MDR-TB) and now the extensively-drug-resistant tuberculosis (XDR-TB) are major challenges for TB elimination. The estimated number of cases of MDR-TB was 687,839 globally in 2019 [3]. According to the Global TB Report, by 2022, Africa will have recorded 2.3% of MDR/XDR-TB cases among new cases and 12% of MDR/XDR-TB cases among those already treated. Tuberculosis causes 1.3 million deaths worldwide [4].

These resistant forms of TB also pose serious social, economic, and healthcare problems to many countries (mainly low and middle-income countries) and their health systems. For example, in 2021, in low and middle-income countries, expenses of TB diagnostic, treatment, and prevention services were estimated at US$5.4 billion. This constitutes a heavy economic and social burden to these countries [3] [5].

Tuberculosis has a significant social and economic impact in Burkina Faso. Socially, the disease contributes to the stigmatisation of patients, which can isolate them and make them reluctant to seek treatment. Economically, despite the National TB Programme's subsidies for patient care, tuberculosis imposes high costs on families due to additional medical expenses and loss of income during illness. In addition, the disease can affect the productivity of workers and have an impact on communities, particularly in rural areas where health infrastructure is often inadequate. (Burkina Ministry of Health)

There are differences in the disease burden between regions and countries. TB occurs in every region of the world, with over 95% of cases and deaths occurring in developing countries [6]. The WHO region with the highest number of new tuberculosis cases was Southeast Asia (46% of all new cases), followed by the African Region (23%) and the Western Pacific Region (18%) [6]. Concerning multidrug-resistant tuberculosis, Eastern European countries, Russia and Central Asian countries, and parts of China have a high rate of MDR-TB infection [7]. However, in a study by Moga *et al.*, the authors reported that the level of MDR-TB in East Africa is higher than in other regions globally [8]. In 2015, the global MDR-TB prevalence in new and previous TB cases was 3.5% and 20.5%, respectively with greater rates in countries of southern regions of Africa [9]. Currently, the spread of drug-resistant tuberculosis is one of the world's major public health problems and several non-mutually exclusive risk factors have contributed to the spread of this form of the disease [10] [11].

A recent systematic meta-analysis review study, including 148 studies with a sample size of 318,430 people [2], reported a very high global prevalence of drug-resistant tuberculosis in 2023, with a global pooled prevalence of multi-drug-resistant TB at 11.6% (95% also reported the highly concerning XDR-TB at a global prevalence of 2.5%).

The effective control and prevention of TB and its resistant forms need rapid diagnostic and appropriate TB treatment regimens application. Thus, WHO endorsed 2008 molecular tests such as Line Probe Assays (LPA) and the automated Cepheid Gene Xpert MTB/RIF system (Cepheid Xpert Inc., Sunnyvale, CA, USA) for rapid screening of TB and drug-resistant TB. Some current WHO-endorsed line probe assays are INNO-LiPA Rif. TB (INNOLIPA; Innogenetics, Zwijndrecht, Belgium), Genotype MTBDR/MTBDR*Plus*, and Genotype MTBDRsl (GTsl; Hain Lifescience, GmbH, Germany) [12].

These molecular tests offer a rapid alternative to conventional bacteriological methods and are a very important alternative for resource-limited countries with limited diagnostic capacity, high prevalence of TB or MDR-TB, and now the

emerging pre-XDR and XDR-TB. For example, West African countries are heavily affected by TB and MDR-TB (6%) in new patients and (35%) in retreatment patients. There have also been important emerging of pre-XDR (35% of MDR in Ghana), the prevalence of which is underestimated by the WHO [13]. In Ghana and Togo, pre-XDR isolates are circulating amongst new patients and XDR-TB.

For these countries, the use of culture is compromised by several difficulties such as culture contamination, no growth in a subculture, or other challenges encountered in the laboratory when performing growth-based drug susceptibility testing [14] [15].

In Burkina Faso, the national resistance surveillance carried out in 2017 showed a prevalence of MDR-TB of 2% and 14% in new and previously treated patients respectively [16]. There is strong evidence (several studies) that the global or regional prevalence of drug-resistant tuberculosis is very high, so health authorities should consider ways to manage and control the disease to prevent further spread of tuberculosis and potential health disasters.

This work aims to contribute to a better estimation of the MDR-TB burden in Burkina Faso, which will guide public health authorities and government agencies (National Tuberculosis Program) to make evidence-based decisions and policies for effective control and prevention.

2. Material and Methods

2.1. Study Population and Sample Collection

The study population included subjects in contact with symptomatic DR-TB cases (the patient's family, colleagues, or staff caring for DR-TB patients, fellow inmates), subjects already treated for TB (failure, relapse, resumption, and other) and smear-positive cases at the 2nd or 3rd month of first-line anti-tuberculosis treatment. All new smear-positive TB cases, *i.e.*, those who had never received antituberculosis treatment or who had received it for less than a month, were included in the study population. All participants were interviewed and a questionnaire was administered to collect socio-demographic and clinical information. Participants were then registered and specimens (spontaneous or induced sputum, gastric tube fluid, bronchoalveolar lavage (BAL), pleural fluid, urine, cerebrospinal fluid (CSF), effusion, and ascites fluid) were collected. All participants were enrolled at the Laboratoire National de Référence des Mycobactéries (LNR-M), Centre National de Lutte Antituberculeuse (CNLAT), Ouagadougou, Burkina Faso.

2.2. Detection of *Mycobacterium tuberculosis* Complex and Rifampicin Resistance by Xpert MTB/RIF

For tuberculosis and rifampicin (one of the main first-line anti-tuberculosis drugs) resistance screening, we used the Gene Xpert MTB/RIF (Cepheid, USA) molecular technique as a first test following the manufacturer's instructions. This test enables simultaneous diagnosis of TB (detection of MTB complex DNA) and

detection of rifampin resistance (detection of *rpoB* gene mutations conferring rifampicin resistance) in less than 2 hours. Briefly, it is an automated test performed by liquefying the samples in 15 ml conical tubes and mixing them with the reagent supplied in the Xpert MTB/RIF kit. Then, using a sterile Pasteur pipette, 2 ml of each liquefied sample was transferred to a cartridge. Finally, the cartridge is placed in one of the pre-programmed GenXpert instrument modules for running.

2.3. *Mycobacterium tuberculosis* Complex and Rifampicin and Isoniazid Resistance Detection in a Subset of Study Samples by the GenoType MTBDR*Plus* V2.0 Test

A subset of 35 rifampicin-resistant TB patients' samples were analyzed by the GenoType MTBDR*Plus* V2 test (Hain Life Sciences, Nehren, Germany). The GenoType MTBDR*Plus* V2 test is a line probe (LPA)˚ assay, manual and comprising the DNA extraction, the amplification, and the hybridization steps.

DNA extraction step

DNA extraction was performed using the GenoLyse kit (Hain Life Sciences, GmbH, Nehren, Germany) according to the manufacturer's instructions. Briefly, frozen pellets were thawed at room temperature and then vortexed. Then 500 µl of each pellet was dispensed into labeled cryotubes and centrifuged for 15 minutes at 4˚C. The supernatants were discarded, and 100 µl of A-lys was added to the pellets in each cryotube, which were then placed in a thermomixer at 95˚C for 5 minutes. 100 µl of the neutralization solution (A-NB) was added to the cryotubes and the mixture was centrifuged for 5 minutes at 4˚C. Finally, DNA extracts were packed into new cryotubes and stored at −20˚C.

Amplification step

Amplification was performed using the Genotype MTBDR*Plus* V2 kit (Hain Life Sciences, GmbH, Nehren, Germany) according to the manufacturer's recommendations. The reaction mixture consisted of 10 µl of mix A; 35 µl of mix B and 5 µl of DNA extract. Ultra-pure nuclease-free distilled water was used as a negative control. Amplification was performed in a GTQ-Cycler 96 thermal cycler (Hain Life Sciences, GmbH, Nehren, Germany). The PCR programme (temperature cycles) was: a first denaturation at 95˚C for 15 minutes, followed by 10 cycles of 30 seconds at 95˚C and 120 seconds at 65˚C and after 30 cycles of 25 seconds at 95˚C, 40 seconds at 50˚C and 40 seconds at 70˚C and a final extension at 70˚C for 8 minutes.

Hybridization step

Hybridization was performed using GenoType MTBDR*Plus* V2 kit according to the manufacturer's instructions. Briefly, 20 µl of denaturation buffer (DEN) was dispensed into the well of a TwinCubator® tray (Hain Lifescience GmbH, Nehren), followed by the addition of 20 µl of each amplicon and the mixture was left at room temperature for 5 minutes to allow denaturation to proceed. Finally, the colored bands on the strips were read and interpreted using the GenoType MTBDR*Plus* V2 card.

2.4. Data Analysis

The data obtained from the collection sheets were entered into Microsoft Excel Office 2007. They were then compiled, analyzed, and interpreted using R Studio 4.3.1 software. We used the Fischer test for statistical tests to compare results and calculate odds ratios and confidence intervals. The significance level is 5%. Graphs were constructed using Excel software.

2.5. Ethical

This study was authorized by the Centre National de Lutte Antituberculose (CNLAT) of Burkina Faso, n˚2023 028 MSHP/SG/DGSP/DPSP/CNLAT. All patients gave informed consent before inclusion in the study.

3. Results

3.1. Xpert MTB/RIF Test Analysis Flowchart from Patient Enrolment

The Xpert MTB/RIF test was used as the first test to screen all the samples included in this study. Of the 500 samples analyzed by the Xpert MTB/RIF test, 33.7% (169/500) [95% IC: 29.7 - 37.9] were positive for TB. Of the 169 cases, 40.83% (69/169) were previously treated patients and 59.17% (100/169) were new cases. The resistance to rifampicin was 40.58% (28/69) in previously treated patients and 13% (13/100) in new cases. Figure 1 shows a flowchart of sample analysis using the Xpert MTB/RIF assay.

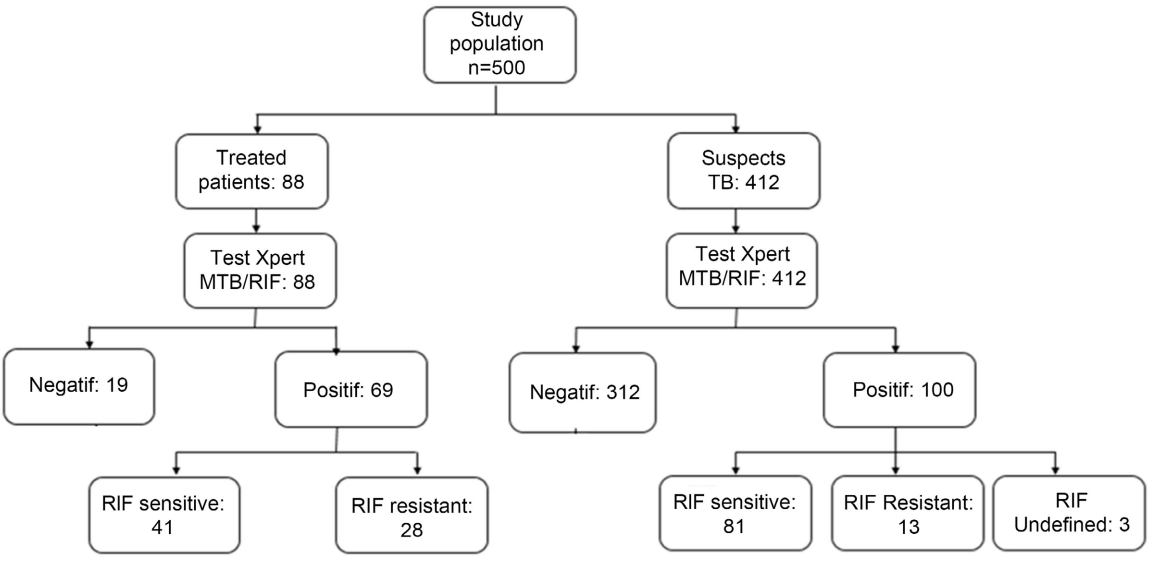

Figure 1. Flowchart of the Xpert MTB/RIF assay for sample analysis.

3.2. Socio-Demographic Characteristics and Geographic Distribution of Tuberculosis Patients Confirmed by Xpert MTB/RIF Assay

Of the 500 suspected TB patients included in this study, 169 were confirmed by

the Xpert MTB/RIF assay by detection of *Mycobacterium tuberculosis* complex DNA. Of the 169 TB-positive cases, male patients were the most common, accounting for 84.0% of cases ($P < 0.05$), with a sex ratio of 5.3. The mean age of included patients was 41.12 ± 14.03 years. Over 55.62% of these patients were between 25 and 45 years of age. The 35 - 45 age group was the most represented, accounting for 31.36% of patients. For males, the [35 - 45] age group was the most affected, with 28.4% of cases. For females, two age groups [25 - 35[and [45 - 55[were the most represented, with 3.55% of cases each (Figure 2).

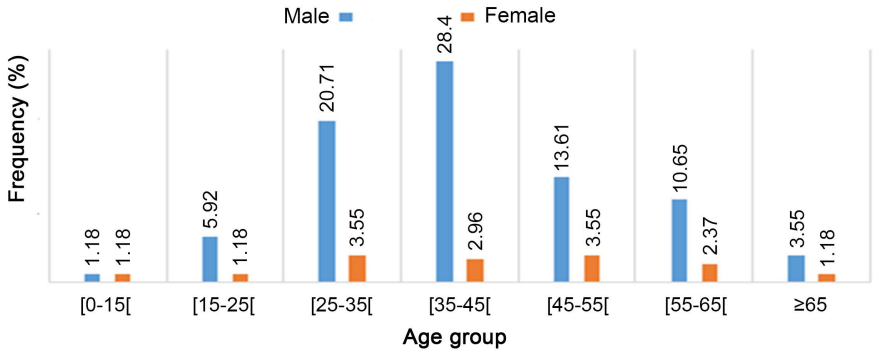

Figure 2. Distribution by gender and age group of TB patients confirmed by Xpert MTB/RIF assay.

The country of residence of TB patients was mainly Burkina Faso, with a significant number of TB patients coming from neighboring Côte d'Ivoire (Ivory Coast) to receive health care in Burkina Faso. Of all patients, 85.80% (145/168) lived in Burkina Faso, 12.43% (21/169) in Côte d'Ivoire, and 1.77% (3/169) in other countries. Considering the life environment of patients, 60.36% lived in urban areas and 39.64 were rural (Table 1).

Table 1. Geographic distribution of TB patients confirmed by Xpert MTB/RIF assay.

Spatial distribution	Numbers	Frequency (%)
Country		
Burkina Faso	145	85.80
Ivory Coast	21	12.43
Other	03	1.77
Total	**169**	**100**
Life environment		
Urban	102	60.36
Rural	67	39.64
Total	**169**	**100**

3.3. Rifampicin Resistance (RR) Tuberculosis and MDR-TB Detected by Xpert MTB/RIF and the Genotype MTBDR*Plus* V2 Assays

Of the 169 TB patients, 41, or 24.26%, were found to have rifampicin-resistant

tuberculosis by the Xpert MTB/Rif test. Rifampicin resistance is a surrogate marker of multidrug resistance, as more than 90% of rifampicin-resistant strains of Mycobacterium tuberculosis complex are also resistant to isoniazid, making them MDR strains. Based on rifampicin resistance, MDR-TB can be predicted by the Xpert MTB/Rif assay with a prevalence of 24.26%.

In previously treated patients (n = 69), the RR TB or MDR-TB (prediction) was 40.58% (28/69), and in new TB cases (n = 100), RR TB or MDR-TB (prediction) was 13% (13/100).

Of the 41 RR-TB or MDR-TB (prediction) detected by the Xpert MTB/RTF assay a subset of 35 samples randomly chosen was analyzed by the Genotype MTBDR*Plus* V2 which allows the detection of resistance to both rifampicin and isoniazid. Genotype MTBDR*Plus* V2 results showed 5.71% (2/35) cases of mono-resistance to rifampicin, 14.28% (5/35) cases of mono-resistance to isoniazid among previously treated cases and 74.29% (26/35) cases of resistance to both rifampicin and isoniazid (detected MDR-TB) including 9 new cases and 17 previously treated cases (Table 2).

Table 2. Drug-resistance of positive patients to *Mycobacterium tuberculosis* complex detected by Xpert MTB/RIF and GenoType MTBDR*Plus* V2 assays in new and previously treated TB cases.

	Drug-Resistance Xpert MTB/RIF assay (N = 169)			GenoType MTBDR*Plus* V2 assay (N = 35)		
Resistance	**New TB cases (n = 100)**	**PTTB cases (n = 69)**	**Total (N = 169)**	**New TB cases (N = 10)**	**PTTB cases (N = 25)**	**Total = 35**
Rifampicin*	13/100 (13%)	28/69 (40.58%)	41/169 (24.26%)	0/10 (0.0)	2/25 (8%)	2/35 (5.71%)
Isoniazid**	NA	NA	NA	0/10 (0.0)	5/25 (20%)	5/35 (14.28%)
Rifampicin + Isoniazid	NA	NA	NA	9/10 (90%)	17/25 (68%)	26/35 (74.28%)

PTTB: Previously treated TB; NA: No Applicable; *concerning detected mono-resistance to rifampicin by GenoType MTBDR*Plus* V2 test; ** concerning detected mono-resistance to isoniazid by GenoType MTBDR*Plus* V2 test.

The results of the two assays, Xpert MTB/RIF versus GenoType MTBDR*Plus* V2, have shown some discrepancies between them in this use in the field. (Table 3).

3.4. Genes Mutations Conferring Rifampicin and Isoniazid Resistance Detected by Genotype MTBDR*Plus* V2

Xpert MTB/RIF and GenoType MTBDR*Plus* V2 principle of genotypic drug resistance detection is based on the detection of drug-resistance associated-mutations in genes such as *rpoB* gene (the resistance determining region) for rifampicin and the *katG* and *inhA* genes for isoniazid resistance. The common resistance-associated mutations are described by several studies and WHO has published a recent catalogue of these mutations. The main circulating mutations detected by the GenoType MTBDR*Plus* test V2 within rifampicin and isoniazid-resistant isolates in Burkina Faso in this study were respectively the *rpoB* gene mutations D516V at 20% (7/35), S531L at 20% (7/35), H526D at 15.29% (5/35) for

rifampicin resistance and *katG* gene mutation S315T1 at 80% (28/35), *inhA* mutations C15T at 5.71% (2/35), T8C at 14.29% (5/35) and T8A at 2.86% (1/35) for the isoniazid resistance (high-level resistance with *katG* mutation S315T and low level of resistance with *inhA* mutations). The details of all the resistance-associated mutations are described in Table 4.

Table 3. Discordances between Xpert MTB/RIF results (N = 35 rifampicin-resistant MTBc isolates) and GenoType MTBDR*Plus* V2 assay results of the same 35 isolates.

Patient status on MTBDR*Plus* V2	Number	Frequency (%)	Comparison of the 2 test results
MTBc+: RIF-/INH-	01	2.86	**Discordance between Xpert and MTBDR*Plus***
MTBc+: RIF+/INH-	02	5.71	RIF+ on Xpert and RIF+INH- on MTBDR*Plus* (RIF mono-resistance)
MTBc+: RIF-/INH+	05	14.28	**Discordance between Xpert and MTBDR*Plus*** (INH mono-resistance)
MTBc+: RIF+/INH+	26	74.28	RIF + and INH + (MDR-TB)
MTBc-	01	2.86	**Discordance between Xpert and MTBDR*Plus*** (not MTBc detected)
Total	35	100	

MTBc +: *Mycobacterium tuberculosis* complex positive; MTBc: *Mycobacterium tuberculosis* complex negative; RIF+: rifampicin-resistant, RIF-: rifampicin-susceptible; INH+: isoniazid-resistant; INH-: isoniazid-susceptible.

Table 4. Prevalence of rifampicin and isoniazid resistance-associated mutations within the subset samples (N = 35) analyzed by the Genotype MTBDR*Plus* V2.

Rifampicin resistance-associated mutations in *rpoB* gene (N = 35)				
WT probes target Region	**Hybridization of mutant probes**	**Mutation or mutation range region**	**Number of strains**	**Frequency (%)**
ΔWT2/3	No hybridization	510 - 517	01	2.86
ΔWT3/4	MUT1 hybridised	D516V	07	20
ΔWT7	MUT2A hybridised	H526Y	04	11.43
	MUT2B hybridised	H526D	05	15.29
	MUT2A hybridised and MUT2B not hybridised	526 - 529	01	2.86
ΔWT8	MUT3 hybridised	S531L	07	20
	MUT3 not hybridised	530 - 533	03	8.57
Isoniazid resistance associated-mutations in *katG* gene (N = 35)				
ΔWT	MUT1 hybridised	S315T1	28	80
Isoniazid resistance associated-mutations in *inhA* gene (N = 35)				
ΔWT1	MUT1 hybridised	C15T	02	5.71
ΔWT2	MUT3A hybridised	T8C	05	14.29
	MUT3B hybridised	T8A	01	2.86

4. Discussion

Of the 500 suspected TB cases, 169 (33.8%) patients were confirmed positive for TB by the Xpert MTB/RIF assay, including 40.83% (69/169) previously treated

patients and 59.17% (100/169) new cases. The sex distribution of TB patients showed 142 male patients and 27 female patients, giving a sex ratio of 5.25.

This high prevalence in men has been reported in other studies conducted in several countries, including Burkina Faso, Morocco, and Senegal [16]-[18].

In Burkina Faso, as in many other countries, men are more exposed to risk factors than women, particularly in terms of their socio-professional activities (e.g., exposure to harmful particles in artisanal gold mining) and their behaviors (smoking, alcohol, and drug use). These exposure factors may therefore partly explain this predominance of infection in men [2].

The results of this study also showed that in Burkina Faso, TB mainly affects young adults during their most productive years, which is supported by other studies in Cameroon [19]. The average age of patients was 41.12 ± 14.03 years, and more than half of tuberculosis cases (55.62%) were between the ages of 25 and 44. The most affected age group was 35 - 45, with 31.36% of cases. However, the age groups most affected in our study relatively differ from those reported in Morocco, where the authors found that 54.88% of patients were aged between 15 and 34 [17]. Therefore, strategic TB control interventions are warranted to specifically target and prevent TB among these age groups.

Regarding the place of residence of TB patients, 85.80% lived in Burkina Faso and 12.43% came from Côte d'Ivoire for treatment. Burkina Faso has a large community of nationals who have immigrated to Côte d'Ivoire, but many of them return to Burkina Faso for treatment when they fall ill, due to the better conditions and the accessibility of quality care.

The incidence of tuberculosis in Côte d'Ivoire, estimated by the WHO at 128 cases per 100,000 inhabitants in 2021, is higher than the incidence in Burkina Faso, estimated at 45 cases per 100,000 inhabitants [6]. As staying in a country with a high incidence of tuberculosis is a risk factor for contracting the disease, Burkinabè migrants to Côte d'Ivoire are at high risk of becoming infected with Mycobacterium tuberculosis complex and then spreading it to their contacts and other people during their stay in Burkina Faso. Moreover, they can start treatment in Côte d'Ivoire and stop traveling in Burkina Faso, and this miss observation of treatment allows drug-resistance development such as MDR-TB. The 2010 study by Saleri *et al.* [20] documented this migration of TB patients from Côte d'Ivoire to Burkina Faso and the development of MDR-TB or XDR-TB with transmission to contacts of these patients in Burkina Faso. Another neighbouring country of Burkina Faso with a significant prevalence of resistant forms of TB is Ghana, where a 2017 study in the Eastern Region found that 32.9% (26/79) of TB cases were MDR-TB [21].

The fight against TB therefore requires transnational strategies and cooperation, as the pathogen does not respect national borders. The national TB control programme of Burkina Faso and those of its neighboring countries need to work together to deal with the threat.

Tuberculosis is a disease of poverty, affecting mainly the underprivileged pop-

ulation with precarious living conditions (promiscuity and ignorance of certain basic rules of hygiene) [22] [23]. In our study, almost 60.36% of patients lived in urban areas and the most affected were workers and farmers with proportions of 23.67% and 23.07% respectively. This high rate of tuberculosis in urban areas has also been reported in studies carried out in Senegal and Morocco [17] [24]. The workers were employed as mechanics, drivers, bricklayers, carpenters, tailors, mechanics, and craftsmen.

The study highlights the alarming prevalence of the MDR-TB in Burkina Faso. Compared to previous studies' data from national surveys, it increased considerably over these recent years in new and mainly previously treated patients to reach 13% and 40.58% (prevalence of MTB-DR predicted based on Xpert MTB/RIF results) respectively.

A first survey study of MDR-TB performed from 2005 to 2006 and published in 2010 by Sangaré *et al.* found MDR-TB to be 3.4% and 50.5% among new cases and previously treated TB patients, respectively [16] [25]. A second study published in 2019 by Diandé *et al.*, using survey data from 2016-2017, reported a prevalence of rifampicin-resistant TB of 2% and 14.5%, of which 83% were MDR-TB in new and previously treated TB patients, respectively [16].

All these data show that MDR-TB has been a major threat to public health in Burkina Faso for many years up to today. This situation is confirmed by the NTP's annual activity reports, which show that MDR-TB among previously treated patients is increasing every year.

These evidence-based results enabled Burkina Faso's National Tuberculosis Programme (NTP) to implement a comprehensive package of laboratory interventions to strengthen TB control from 2016 to 2018, leading to a significant improvement in all indicators. For example, based on these interventions, respectively in 2016 and 2018, bacteriologically confirmed cases increased from 67% to 71%, new and relapse TB cases notified tested by Xpert MTB/RIF increased from 18% to 46%, and notified MDR-TB cases on the estimated number of MDR-TB cases increased from 43% to 78% [26]. Despite these satisfactory results in all indicators achieved by the NTP, the results of this study show that it is still necessary and urgent to maintain these gains, strengthen control of MTB-TB, and innovate strategies and algorithms.

Improving diagnostic and treatment algorithms must include XDR-TB diagnosis, especially for any MDR-TB case, as the circulation of XDR-TB in Burkina Faso cannot be excluded. Although the study by Diandé *et al.* in 2019 did not report any cases of XDR-TB or pre-XDR-TB in Burkina Faso, all countries with diagnostic capacity have reported cases of XDR-TB [27]. For example, developed countries where MDR-TB is not a public health problem report cases of XDR-TB. The United States reported 100 cases of multidrug-resistant TB in 2023, with pre-XDR-TB and XDR-TB still rare, with 15 cases of pre-XDR-TB and one case of XDR-TB reported in 2023 [28]. Furthermore, the study by Saleri *et al.* [20], reported 2 cases (5.9%) of XDR-TB among MDR-TB patients in Burkina Faso. How-

ever, these patients had already started second-line treatment.

Another finding of this study is the agreement of the two WHO-recommended molecular assays, Xpert MTB/RIF and GenoType MTBDR*Plus* V2, for routine TB diagnosis. These tests detect *Mycobacterium tuberculosis* complex bacteria and their resistance to rifampicin (Xpert MTB/RIF) or rifampicin plus isoniazid (GenoType MTBDR*Plus* V2) based on the detection of genes mutations-conferring resistance.

However, there were a few discrepancies between Xpert MTB/RIF and GenoType MTBDR*Plus* V2 in the results of certain patients. One patient who was positive for TB by Xpert MTB/RIF was negative for TB by the MTBDR*Plus* V2 assay. One patient positive for rifampicin-resistant TB by Xpert MTB/RIF was rifampicin-sensitive by the MTBDR*Plus* V2 assay and five patients positive for rifampicin-resistant TB by Xpert MTB/RIF were rifampicin-sensitive by the MTBDR*Plus* V2 assay but isoniazid-resistant. Other studies already reported these kinds of results discrepancies between the two tests [16] or demonstrated that the sensitivity of MTBDR*Plus* V2 for isoniazid resistance detection was less than 100% (77.4% of sensitivity among isoniazid-resistant isolates and 94.3% of sensitivity among MDR-TB isolates in the study of Moga *et al.*, 2023) [8]. The implications of these discrepancies are that the use of one test or another can lead to false positives or negatives with consequences in terms of inappropriate treatment.

The reasons that may explain these discrepancies are the probable inferior sensitivity of the MTBDRPlus V2 test compared to the Xpert MTB/RIF test and operator error. The 2 tests use different probes for the targets. MTBDR*Plus* V2 (MTBDR*Plus* V2 version 2) is an optimisation of version 1, to increase its sensitivity a version 3 may be required. In addition, the MTBDR*Plus* V2 is performed manually, which can lead to operator error compared to the automated Xpert MTB/RIF. Therefore, the performance of these 2 tests, mainly the MTBDR*Plus* V2, needs to be evaluated and optimised in Burkina Faso.

The common mutations conferring resistance to rifampicin and isoniazid targeted by the two tests are mainly located in the *rpoB* gene for rifampicin resistance and in the *katG* and *inhA* genes for isoniazid resistance. An updated list of these mutations is published by WHO. In this study, the most prevalent mutations detected in the MTBc isolates of Burkina Faso are D516V (20%), S531L (20%), H526D (15.29%), and H526Y (11.43%) in *rpoB* gene also confirmed by many studies as the most common *rpoB* gene mutations [29] [30].

However, there are geographical differences in the prevalence or types of these *rpoB* gene mutations due to various factors such as treatment regimens or other geographical differences [29] [30]. For example, in South Africa, the most prevalent *ropB* gene mutations conferring rifampicin resistance reported by the systematic review of Traoré *et al.* in 2023 were S450L (80%), Ile 491 Phe (8%) and L430P (8%) [31].

For isoniazid resistance, the most prevalent mutations reported in this study corroborated by several worldwide studies were mainly the *katG* gene mutation

S315T1 with 80% [31].

However, there are other mutations conferring isoniazid resistance such as S315T2 in *katG* gene [29] [30] and *inhA* gene mutations T8C, C15T, and T8A respectively 14.29%, 5.71%, and 2.86% of prevalence in this study.

The geographical variation of MBTc drug resistance-conferring mutations highlights the importance of monitoring these mutations for updating and optimizing molecular tests.

5. Conclusion

The results of this study provide evidence-based confirmation that MDR-TB is a major concern in Burkina Faso and a challenge for TB elimination. As rifampicin resistance is a surrogate marker for MDR-TB, the predicted global prevalence of MDR-TB was 24.26% (41/169), including 28 cases of MDR-TB among previously treated TB patients (40.58%, 28/69) and 13 cases of MDR-TB among new TB cases (13%, 13/100). There is an urgent need for the country to take immediate action and plan strategies to control multidrug-resistant tuberculosis. These measures must include expanding the use of the Xpert/MTB-Rif and MTBDR*Plus* tests to detect resistance to rifampicin and isoniazid in all patients with suspected TB and to diagnose XDR-TB in all patients with MDR-TB.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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