Journal of Biosciences and Medicines, 2026, 14(6), 215-230
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Detection of Mutations in Genes Associated with Multidrug Resistance in the *Mycobacterium tuberculosis* Complex among HIV/Tuberculosis Co-Infected Patients in N'Djamena, Chad

Abakar Oumar Mahamat[1*], Abderrazzack Adoum Fouda[1,2,3], Richard Bongo Naré Ngandolo[4], Hidir Tidjani Abakar[1], Ahmadou Oumarou[5], Djasra Frederic[5], Lamireou Didi[4], Oumalkher Youssouf Adam[3], Ahmat Mahamat Ahmat[6], Djamalladine Mahamat Doungous[7], Hassoumi Manah[8], Abdoullahi Hissein Ousman[1], Mahamat Nour Aguid[6], Oumar Abdelhadi[5]

[1]Laboratoire de Recherche, Diagnostic et Expertise Scientifique (Labo-REDES), Faculté des Sciences de la Santé Humaine (FSSH), Université de N'Djamena, N'Djamena, Tchad
[2]Faculté des Sciences et de la Technologie, Université Toumaï, N'Djamena, Tchad
[3]Ministère de la Santé Publique et de la Prévention, N'Djamena, Tchad
[4]Institut de Rechercher en Elevage pour le Développement (IRED), N'Djamena, Tchad
[5]Laboratoire National de Reference, Programme National de Lutte Contre la Tuberculose (LNR/PNT), N'Djamena, Tchad
[6]Centre d'Appui-Psycho-Médico-Social (APMS), N'Djamena, Tchad
[7]Département des Sciences Biomédicales et Pharmaceutiques, Institut National Supérieur des Sciences et Techniques d'Abéché, Abéché, Tchad
[8]Centre Hospitalier Universitaire de l'Amitié Tchad-chine (CHU-ATC), N'Djamena, Tchad
Email: *abakaromc@gmail.com

How to cite this paper: Mahamat, A.O., Fouda, A.A., Ngandolo, R.B.N., Abakar, H.T., Oumarou, A., Frederic, D., Didi, L., Adam, O.Y., Ahmat, A.M., Doungous, D.M., Manah, H., Ousman, A.H., Aguid, M.N. and Abdelhadi, O. (2026) Detection of Mutations in Genes Associated with Multidrug Resistance in the *Mycobacterium tuberculosis* Complex among HIV/Tuberculosis Co-Infected Patients in N'Djamena, Chad. *Journal of Biosciences and Medicines*, **14**, 215-230.
https://doi.org/10.4236/jbm.2026.146015

Received: March 21, 2026
Accepted: June 15, 2026
Published: June 18, 2026

Abstract

Introduction: HIV/tuberculosis (HIV/TB) co-infection remains a major public health issue in countries with a high tuberculosis burden. The emergence of *Mycobacterium tuberculosis* strains resistant to anti-tuberculosis drugs further complicates the management of co-infected patients, particularly among immunocompromised individuals. This study aimed to detect the mutations in gene associated with multidrug resistance in *Mycobacterium tuberculosis* among HIV/TB co-infected patients in N'Djamena. **Methodology:** A descriptive and analytical cross-sectional study was conducted among HIV/TB co-infected patients followed in treatment centers (APMS and CHU-ATC) in N'Djamena. Sputum samples were analyzed at the IRED and National Reference Laboratory/PNT using molecular techniques: Xpert MTB/RIF for the detection of mutations associated with rifampicin resistance and Xpert MTB/XDR for the detection of mutations associated with multidrug resistance (isoniazid, fluoroquinolones, etc.). Data were analyzed using SPSS version 26. **Re-**

sults: A total of 97 HIV/TB co-infected patients were included in the study. The majority of patients were male, with a predominance of the 25 - 35 years age group. Rifampicin resistance was observed in 4.12% of patients, while resistance to isoniazid and fluoroquinolones was 9.27% and 2.06%, respectively. Regarding resistance profiles, rifampicin monoresistance accounted for 1.03%, isoniazid monoresistance for 6.18%, and fluoroquinolone monoresistance for 1.03%. Multidrug-resistant tuberculosis, defined as simultaneous resistance to rifampicin and isoniazid, was found in 3.09% of HIV/TB co-infected patients. Among these, one sample showed additional fluoroquinolone resistance mutations (*gyrA* and *gyrB*), corresponding to pre-XDR TB (1.03%). **Conclusion:** This study highlights a non-negligible frequency of multidrug resistance to anti-tuberculosis drugs among HIV/TB co-infected patients in N'Djamena. These findings emphasize the importance of rapid molecular diagnosis to improve therapeutic management and limit the spread of resistant *Mycobacterium tuberculosis* strains in Chad.

Keywords

HIV/TB Co-Infection, Multidrug Resistance, Rifampicin, Isoniazid, N'Djamena-Chad

1. Introduction

Tuberculosis (TB) remains a major global public health problem, particularly in low-resource countries, where it is often aggravated by co-infection with the human immunodeficiency virus (HIV). HIV-induced immunosuppression worsens the clinical prognosis and complicates therapeutic management [1].

Despite progress achieved in diagnosis and treatment, the increasing incidence of drug-resistant tuberculosis seriously compromises treatment success. In a worrying evolving dynamic, inadequate patient management, treatment interruption, direct transmission of resistant strains, or spontaneous genetic mutations in mycobacteria (particularly in the *katG* and *rpoB* genes) may lead to a worsening resistance profile [2]. Consequently, increasingly complex resistant strains of *Mycobacterium tuberculosis*, particularly multidrug-resistant tuberculosis (MDR-TB), are emerging worldwide [3].

MDR-TB is defined as an infection caused by a mycobacterium belonging to the *Mycobacterium tuberculosis* complex that is resistant to at least isoniazid and rifampicin, the two most powerful first-line anti-tuberculosis drugs [2] [4]. In 2018, the World Health Organization (WHO) estimated that approximately half a million new cases of rifampicin-resistant TB occurred worldwide, of which 78% were MDR-TB cases, highlighting rifampicin resistance as a poor prognostic factor [4] [5].

According to the WHO in 2024, approximately 57,000 people in the African region developed multidrug-resistant or rifampicin-resistant tuberculosis, repre-

senting 15% of the global burden [6]. Chad is not spared from this burden, where the rate of drug-resistant tuberculosis in the general population remains significant [7]. The incidence of multidrug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB) is estimated at 2.4 cases per 100,000 inhabitants, corresponding to approximately 420 estimated MDR-TB cases. The proportion of MDR-TB is estimated at 1.5% among new cases and 3.7% among previously treated cases [8]. According to the National Tuberculosis Control Program (PNT), the number of laboratory-confirmed drug-resistant TB cases does not reach half of the expected cases [9].

Among people living with HIV (PLHIV), the management of MDR-TB is even more complex due to the combined challenges associated with immunosuppression. Early identification of MDR-TB cases among PLHIV, adaptation of therapeutic strategies, and control of disease transmission remain constant concerns for clinicians [4] [5].

However, despite the strong interaction between HIV and tuberculosis, specific data on multidrug resistance among HIV/TB co-infected patients remain limited. Several studies have highlighted that fewer than half of sub-Saharan African countries have reliable data on multidrug-resistant TB, reflecting a substantial gap in surveillance and research [10].

In the Chadian context, where the dual burden of tuberculosis and HIV remains high [11] [12], epidemiological data on multidrug-resistant tuberculosis among HIV/TB co-infected patients are particularly scarce or even nonexistent at the national level, despite the National Tuberculosis Control Program's recommendation to use Xpert MTB/RIF in all sites equipped with GeneXpert devices [13] [9]. This gap constitutes a major obstacle to the development of appropriate management strategies. Therefore, the present study aimed to detect the mutations in genes associated with multidrug resistance to anti-tuberculosis drugs in the *Mycobacterium tuberculosis* complex among HIV/TB co-infected patients in N'Djamena, Chad.

2. Methods

2.1. Study Setting

This was a cross-sectional, prospective, and analytical study conducted from December 2024 to November 2025 among people living with HIV (PLHIV) followed in two HIV care centers: the *Centre d'Appui Psycho-Médico-Social* (APMS) and the *Centre Hospitalier Universitaire de l'Amitié Tchad-Chine* (CHU-ATC) in N'Djamena. Sample analyses were performed at the laboratory of the *Institut de Recherche en Élevage pour le Développement* (IRED) and at the *Laboratoire National de Reference, Programme National de lutte contre la Tuberculose* (LNR/PNT), in N'Djamena.

2.2. Study Population

The study population consisted of HIV/TB co-infected patients of all ages and

both sexes consecutively admitted to the study sites during the study period.

2.3. Participant Recruitment Procedure

Participants were consecutively recruited between December 2024 and November 2025 from the two HIV care centers included in the study (APMS and CHU-ATC in N'Djamena). All people living with HIV who presented with clinical or microbiological suspicion of pulmonary tuberculosis during the study period were systematically assessed for eligibility.

The diagnosis of pulmonary tuberculosis was confirmed by the detection of the *Mycobacterium tuberculosis* complex in sputum samples using the Xpert MTB/RIF assay. Only patients with bacteriologically confirmed HIV/pulmonary tuberculosis co-infection who met the inclusion criteria were enrolled in the study.

A total of 530 patients were screened for eligibility. Of these, 433 were excluded because they did not have bacteriologically confirmed pulmonary tuberculosis and/or did not meet the inclusion criteria. Ultimately, 97 participants met the eligibility criteria and were included in the final analysis.

When an Xpert MTB/RIF or Xpert MTB/XDR result was invalid, indeterminate, or associated with a technical error, the test was repeated using the same specimen whenever possible. Only valid results obtained after repeat testing were retained for the final analysis.

2.4. Inclusion Criteria

Patients with confirmed HIV/TB co-infection who provided free and informed consent were systematically included in the study.

2.5. Exclusion Criteria

HIV/TB co-infected patients who did not provide consent were not included in the study. Patients already screened and/or registered during the study period were not re-included in order to avoid duplication of data.

2.6. Variables

The variables recorded in the data collection form for co-infected patients included sociodemographic characteristics such as age, sex, and marital status.

2.7. Sampling Procedure

Sample collection involved sputum and blood specimens from PLHIV included in the study. Sociodemographic information and co-infection data were recorded using a pre-established technical form. Sputum samples were collected from each patient for the detection of Acid-Fast Bacilli (AFB). All sputum samples positive by microscopy were subsequently analyzed to confirm tuberculosis diagnosis and detect the *rpoB* gene associated with rifampicin resistance using the GeneXpert MTB/RIF molecular technology. Thereafter, the Xpert MTB/XDR assay was used for the detection of multidrug-resistant tuberculosis (MDR-TB). To ensure ano-

nymity, samples were identified using codes such as E1, E2, E3… E(n).

2.8. Laboratory Analysis

2.8.1. Diagnosis of HIV Infection

Serological diagnosis of HIV infection was performed according to the Chadian national algorithm, based on WHO strategy 2, involving three sequential rapid tests: Determine HIV-1/2, SD Bioline HIV-1/2 3.0, and CHEMBIO HIV-1/2 STAT-PAK.

The Determine HIV-1/2 test was used for the qualitative detection of anti-HIV-1 and HIV-2 antibodies. Samples positive with Determine HIV-1/2 were further tested using SD Bioline HIV-1/2 3.0 to differentiate HIV types (HIV-1, HIV-2, or HIV-1/2). Positive results from the second test were confirmed using CHEMBIO HIV-1/2 STAT-PAK. All procedures were strictly performed according to the manufacturer's recommendations.

2.8.2. Determination of CD4 T Lymphocyte Count

CD4 count quantification was performed using the VISITECT® CD4 Advanced Disease rapid test. The VISITECT CD4 Advanced Disease Rapid Test is an immunochromatographic and manually operated semi-quantitative assay used for the estimation of CD4 protein expression on the surface of CD4+ T cells in human whole blood (capillary or EDTA venous blood), indicating whether the CD4 level is above or below 200 cells/μL in previously diagnosed HIV patients.

2.8.3. Diagnosis of Tuberculosis Infection

Microscopic Examination of Sputum: Microscopic examination was performed on smears prepared from sputum samples. Detection of AFB was carried out using Ziehl-Neelsen staining [14].

All sputum samples that tested positive by smear microscopy were selected for molecular detection of drug resistance-associated mutations using the GeneXpert platform.

Decontamination Procedure: All sputum samples were decontaminated using the Kubica method [15]. This method involves the use of a sterile solution containing 4% sodium hydroxide (NaOH) and 2.9% sodium citrate, followed by 0.5% N-acetyl-L-cysteine as a decontaminating agent and phosphate buffer (pH 6.8) as a washing solution. Decontamination helps eliminate or reduce contaminating microorganisms present in the sample, thereby enabling reliable microbiological analysis and detection of tuberculosis bacilli. The presence of Acid-Fast Bacilli (AFB) was confirmed after Ziehl-Neelsen staining of smears prepared from culture colonies.

Molecular Diagnosis of Anti-Tuberculosis Drug Resistance: All sputum samples with positive smear microscopy were selected for molecular analysis of resistance mutations using GeneXpert technology [15]. This is an automated cartridge-based test integrating DNA extraction and real-time polymerase chain reaction (PCR) amplification performed on the GeneXpert platform. The test allows

detection of *Mycobacterium tuberculosis* complex DNA in raw sputum or concentrated sediments prepared from spontaneous or induced sputum samples. In samples where the *Mycobacterium tuberculosis* complex (MTB complex) was detected, the Xpert MTB/RIF assay also identified mutations associated with rifampicin resistance in the *rpoB* gene. The results indicated the presence or absence of *Mycobacterium tuberculosis* complex, the presence or absence of rifampicin resistance, and a semi-quantitative estimation of bacillary load (high, medium, low, or very low). Negative tests for *M. tuberculosis* associated with internal control failure were considered invalid [16] [17].

For the molecular diagnosis of multidrug-resistant tuberculosis, the Xpert MTB/XDR assay was used. The Xpert MTB/XDR assay, performed on the GeneXpert Instrument Systems, is a nested real-time polymerase chain reaction (PCR) in vitro diagnostic test for the detection of extensively drug-resistant (XDR) *Mycobacterium tuberculosis* complex DNA in unprocessed sputum samples, concentrated sediments prepared from sputum, or BD™ Mycobacterial Growth Indicator Tube (MGIT™) culture. In specimens where MTB is detected, the assay can also identify mutations associated with resistance to isoniazid (INH) in the *katG* and *fabG1* genes, the *oxyR-ahpC* intergenic region, and the *inhA* promoter; ethionamide (ETH) resistance associated with *inhA* promoter mutations only; fluoroquinolone (FLQ) resistance associated with mutations in the *gyrA* and *gyrB* quinolone resistance-determining regions (QRDR); and second-line injectable drug (SLID) resistance involving amikacin (AMK), kanamycin (KAN), or capreomycin (CAP), associated with mutations in the *rrs* gene and the *eis* promoter region [18].

2.9. Statistical Data Analysis

Data collected from the survey forms were compiled and entered into Microsoft Excel spreadsheets and subsequently analyzed using IBM SPSS version 26 software.

2.10. Ethical Considerations

Each participant was informed about the nature, objectives, and importance of the study. Free and informed consent was obtained either orally or in writing, and confidentiality of the data was strictly maintained. Biomedical analyses were performed free of charge, and results were processed anonymously. The study received approval from the National Bioethics Committee of Chad (Ref: No. 063/MESRSFP/SE/SG/CNBT/SG/2025) and authorization from the competent health authorities (Authorization from the Ministry of Public Health: Ref No. 0403/MSP/SE/SG/BACS/2025).

3. Results

3.1. Sociodemographic Characteristics of Study Participants

A total of 530 HIV-positive patients were recruited during the study period, of

whom 97 were co-infected with HIV/TB, corresponding to a prevalence of 18.3%. Among the 97 HIV/TB co-infected patients included in this study, 61 (62.9%) were male and 36 (37.1%) were female. The mean age of the patients was 38 years, with extremes ranging from 14 to 62 years. Most patients were married (46; 47.4%), followed by single individuals (29; 29.9%). In our study, 31.9% of co-infected patients had a CD4 count below 200 at enrollment (Table 1).

Table 1. Sociodemographic characteristics and clinical variables of study participants (N = 97).

Characteristics	VIH/TB (N)	Frequency (%)
Sexe		
Homme	61	62.9%
Femme	36	37.1%
Age group		
[00 - 18[	2	2.1%
[18 - 25[	14	14.4%
[25 - 35[	35	36.1%
[35 - 45[	29	29.9%
[45 - 55[	9	9.3%
[55 - 65]	8	8.2%
Marital status		
Single	29	29.9%
Divorced	15	15.5%
Married	46	47.4%
Widowed	7	7.2%
Antiretroviral treatment at enrollment		
Yes	58	59.8%
No	39	40.2%
Anti-TB treatment at enrollment		
Yes	34	35.1%
No	63	64.9%
CD4 cell count at enrollment		
<200 cells/µL	31	31.9%
≥200 cells/µL	52	53.7%
Not tested	14	14.4%

The study flow diagram shows that all 97 samples included in the study completed the entire analytical process without any sample loss (Figure 1). Following the decontamination step, all samples (97/97) were analyzed using the Xpert MTB/RIF assay and subsequently tested with the Xpert MTB/XDR assay. There-

fore, the results obtained are based on the total number of samples initially included in the study.

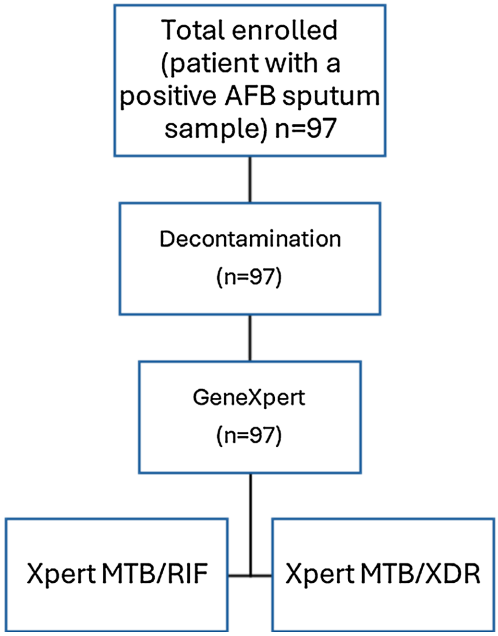

Figure 1. Study Diagram: Samples undergoing (n = 97).

3.2. Identification of Genes Conferring Resistance to Rifampicin and Isoniazid

Frequency and mutation profiles

Among the 97 samples analyzed, 11 patients harbored mutations in genes associated with Mycobacterium tuberculosis resistance to at least one anti-tuberculosis drug, representing a prevalence of 11.34%.

Mutations in the *rpoB* gene conferring rifampicin resistance were identified in 4 out of 97 samples (4.12%). Mutations in genes associated with isoniazid resistance (*inhA* or *katG*) were observed in 9 samples (9.27%). Mutations associated with fluoroquinolone resistance (*gyrA* and *gyrB*) were detected in 2 samples (2.06%) (Table 2).

Regarding resistance profiles, rifampicin monoresistance accounted for 1.03%, isoniazid monoresistance for 6.18%, and fluoroquinolone monoresistance for 1.03% (Table 2).

Multidrug resistance (MDR), defined as resistance to both rifampicin and isoniazid, was detected in 3 samples (3.09%). Additionally, one sample (1.03%) showed combined resistance to rifampicin, isoniazid, and fluoroquinolones (Table 2).

The distribution of resistance patterns among diagnosed cases according to sex and age group is presented in Table 3.

The analysis of Table 4 shows that 7/11 (63.6%) of patients with resistance have a CD4 count below 200 cells/µL, reflecting severe immunosuppression in this group.

Table 2. Drug-susceptibility test results using Xpert MTB/RIF and Xpert MTB/XDR (n = 97).

Xpert MTB/RIF - Xpert MTB/XDR		Number (n)	Percentage (%)
Drug resistance profile by case			
RIF	Resistant	4	4.12%
	Sensitive	93	95.87%
	Indeterminate	0	0.0%
INH	Resistant	9	9.27%
	Sensitive	88	90.72%
	Indeterminate	0	0.0%
FLQ	Resistant	2	2.06%
	Sensitive	95	97.93%
	Indeterminate	0	0.0%
AMK	Resistant	0	0.0%
	Sensitive	96	98.97%
	Indeterminate	1	1.03%
KAN	Resistant	0	0.0%
	Sensitive	96	98.97%
	Indeterminate	1	1.03%
CAP	Resistant	0	0.0%
	Sensitive	96	98.97%
	Indeterminate	1	1.03%
ETH	Resistant	0	0.0%
	Sensitive	97	100%
	Indeterminate	0	0.0%
Monoresistance			
	RIF	1	1.03%
	INH	6	6.18%
	FLQ	1	1.03%
Multidrug-resistance			
MDR	RIF + INH	2	2.06%
Pre-XDR	RIF + INH + FLQ	1	1.03%
Total (MDR)		3	3.09%

Table 3. Distribution of resistance by type of resistance, sex, and age group (N = 97).

Resistance type / Age group/Sex	Mono-resistance (n = 8)			Multidrug-resistance (n = 3)		Sensible (n = 86)	Total (n = 97)
	RIF n (%)	INH n (%)	FLQ n (%)	RIF + INH n (%)	RIF + INH + FLQ n (%)		
Sex							
Male	0 (0.0%)	5 (5.15%)	1 (1.03%)	2 (2.06%)	1 (1.03%)	52 (53.6%)	61 (62.9%)

Continued

Female	1 (1.03%)	1 (1.03%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	34 (35.05%)	36 (37.1%)
Age group							
[0 - 18[	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (2.1%)	2 (2.1%)
[18 - 25[	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	14 (14.4%)	14 (14.4%)
[25 - 35[	1 (1.03%)	1 (1.03%)	0 (0.0%)	1 (1.03%)	0 (0.0%)	32 (32.9%)	35 (36.1%)
[35 - 45[	0 (0.0%)	3 (3.09%)	1 (1.03%)	1 (1.03%)	1 (1.03%)	23 (23.7%)	29 (29.9%)
[45 - 55[	0 (0.0%)	2 (2.06%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (7.21%)	9 (9.3%)
[55 - 65]	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (8.2%)	8 (8.2%)

Table 4. Baseline CD4 cell count of all patients at enrollment.

CD4 cell count at enrollment	Patients with drug-resistant tuberculosis	Percentage
<200 cells/μL	7	63.6%
≥200 cells/μL	3	27.3%
Not tested	1	9.1%
Total	11	100%

4. Discussion

In this study, the presence of gene mutations in *Mycobacterium tuberculosis* associated with resistance to various anti-tuberculosis drugs among HIV/TB co-infected patients was investigated using the Xpert MTB/RIF and Xpert MTB/XDR assays.

Mutations in the *rpoB* gene responsible for rifampicin resistance were detected in 4 patients, corresponding to a rate of 4.12% (4/97). This result is comparable to those reported in South Africa by Nazir *et al.* (4.9% in 2012) [19], in Chad by Ossoga *et al.* (5.21% in 2012) [7], and in Nigeria by Nwalozie *et al.* and Ibrahim *et al.* (5.42% and 4.2% in 2015 and 2018, respectively) [20] [21]. However, other African studies have reported higher proportions than in the present study, such as Ogumbo *et al.* in Kenya (6.7% in 2022) [22] and Atemie *et al.* in Nigeria (9.4% in 2024) [23]. These geographical variations may be influenced by differences in access to rapid molecular diagnostics (such as GeneXpert MTB/RIF), access to medications, treatment adherence, and previous anti-tuberculosis treatment history. The presence of rifampicin resistance among co-infected patients is clinically significant, as it complicates management, increases the risk of treatment failure, and may contribute to the transmission of resistant strains in vulnerable populations.

Regarding isoniazid resistance, mutations were detected in 9 patients, corresponding to a rate of 9.27%. This rate is broadly comparable to findings reported in Nigeria by Lana *et al.* in 2012 (9.3%) [24] and in Cameroon by Merker *et al.* in 2021 (7.4%) [25], although it varies depending on geographical context and study populations. Lower rates have been reported in other studies, such as Brian *et al.*

in Malawi (0.3%) [26], whereas higher levels were reported by Ossoga *et al.* in Chad (12.8%) [7]. Despite the emergence of resistance, isoniazid remains a key drug used both in first-line treatment regimens and in preventive therapy (TPT). This variability may be explained by several factors, including differences in access to rapid diagnostics, treatment adherence, and circulation of resistant strains in certain regions.

For fluoroquinolone resistance (FLQ), mutations were detected in 2 patients, corresponding to a rate of 2.06%. This rate appears relatively low compared to that reported by Mamuda *et al.* in Nigeria in 2026 (12.5%) [27], but remains lower than values described in 2024 by Nehru *et al.* in India (5.12%) [28] and Che *et al.* in China (4.6%) [29]. This difference with highly populated countries may be explained by higher selective pressure due to more widespread and sometimes inappropriate use of fluoroquinolones, both for tuberculosis and other bacterial infections, thereby promoting the emergence of resistant strains. Moreover, differences in surveillance systems and access to drug susceptibility testing may also contribute to these variations. The relatively low rate observed in this study suggests that these drugs remain largely effective in our setting, particularly as second-line treatment options [30].

Among the analyzed samples, rifampicin monoresistance was observed in 1.03% of cases, isoniazid monoresistance in 6.18%, and fluoroquinolone monoresistance in 1.03%. This result is partially similar to that reported in Chad by Awa *et al.* in 2017, where rifampicin monoresistance (5.4%) was higher than isoniazid resistance (13.5%) [13]. However, it differs from findings reported in Nigeria by Lana *et al.* in 2012 (rifampicin 2.8%, isoniazid 1.4%) [24], by Barnett *et al.* in Malawi (rifampicin 2.9%, isoniazid 0.3%) [26], and by Chan *et al.* in South Korea in 2012 (rifampicin 9%, isoniazid 4.54%) [31].

Rifampicin monoresistance is relatively rare. However, in this study, 1.03% of patients showed rifampicin monoresistance. The higher proportion of isoniazid resistance (6.18%) among HIV/TB co-infected patients may be explained by immunosuppression and treatment adherence difficulties in these patients. These resistances inevitably require second-line anti-tuberculosis drugs, which are costly in a resource-limited country such as Chad.

Mutations in both rifampicin resistance (*rpoB*) and isoniazid resistance (*inhA* or *katG*) genes of *Mycobacterium tuberculosis*, defining multidrug resistance (MDR), were detected in 3 samples (3.09%). This rate is close to those reported by Ouédraogo *et al.* in Burkina Faso (3.8% in 2014) [32], Lana *et al.* in Nigeria (3.6%) [24], Barnett *et al.* in Malawi (3.8%) [26], Weyer *et al.* in South Africa (3.4% in 2007) [33]. However, it is lower than those reported by Doungous *et al.* in Chad (5,5% in 2026) [34], by Sied *et al.* in Ethiopia (20% in 2023) [35] and by Chan *et al.* in Seoul, South Korea (15.9% in 2021) [31], but higher than the 0.9% reported in Chad by Dlinga *et al.* in 2023 [36]. These variations may be explained by differences in access to rapid molecular diagnosis, drug availability, treatment adherence, and previous treatment history. The presence of resistance among co-in-

fected patients is clinically significant, as it complicates management, increases the risk of treatment failure, and may contribute to transmission of multidrug-resistant strains in vulnerable populations.

Among these, one sample showed additional fluoroquinolone resistance mutations (*gyrA* and *gyrB*), corresponding to pre-XDR TB (1.03%). This proportion, although low, remains concerning as it reflects the circulation of highly resistant strains within a vulnerable population, often exposed to difficulties in treatment adherence, prior treatment history, and drug interactions, particularly in the context of HIV–tuberculosis. According to the WHO, pre-XDR forms represent a major public health problem requiring early diagnosis and strengthened surveillance in order to reduce their spread and improve clinical outcomes [5].

A clear male predominance was observed (9M/2F), consistent with findings reported in Ethiopia by Hirpa *et al.* in 2013 [37], in the DRC by Misombo *et al.* in 2016 [38], in Gabon by Kombila *et al.* in 2021 [39], and in Chad by Dlinga *et al.* in 2023 [36]. Other studies, however, have reported female predominance, such as in Congo by Okemba-Okombi *et al.* in 2020 [40] and in Ethiopia by Desissa *et al.* in 2018 [41]. This discrepancy may be partly explained by the composition of our study sample, which included a higher proportion of male participants. Indeed, sex distribution within a study population can strongly influence the observed sex ratio.

In this study, the 35 - 45-year age group was the most affected by multidrug resistance, accounting for more than 60% of cases. This result is similar to that of Kombila *et al.* in Gabon in 2021, who reported 25 - 45 years in 73.1% of cases [39], and LaFreniere *et al.* in Canada in 2020, who reported 35 - 44 years in 41.3% of cases [42]. However, it differs from findings in the DRC by Misombo *et al.* in 2016 (16 - 36 years, 61%) [38] and in Congo by Okemba-Okombi *et al.* in 2020 (20 - 29 years, 53.84%) [40]. This may be explained by the higher exposure of this age group to risk factors, including repeated antibiotic use and poor treatment adherence, thereby favoring the emergence of multidrug-resistant strains.

In our study, CD4 count below 200 cells/μL observed in patients co-infected with HIV/TB is an important marker of severe immunosuppression and is frequently associated with poor clinical outcomes, particularly in the presence of resistance to anti-tuberculosis drugs. In this study, among the 11 patients presenting resistance to at least one anti-tuberculosis drug, 7 had a CD4 count below 200 cells/μL, corresponding to a rate of 63.6%. This proportion may reflect advanced immunosuppression, which could favor the persistence or emergence of drug-resistant forms of tuberculosis. Several studies have shown that HIV/TB co-infected patients with CD4 counts < 200 cells/μL experience higher mortality and poorer treatment outcomes compared to those with higher CD4 levels. For instance, a study conducted in Benin reported significantly higher mortality among patients with CD4 counts < 200 cells/mm^3 compared to those with CD4 counts > 200 cells/μL [43]. Similarly, among patients with multidrug-resistant tuberculosis, low CD4 counts, particularly ≤ 100 cells/μL, were strongly associated with reduced

survival and increased risk of death [44]. These findings suggest that severe immunosuppression contributes to worsening tuberculosis progression and may promote treatment failure as well as the development of resistance to anti-tuberculosis drugs.

This study has several limitations. The relatively small sample size may have limited the precision of the estimates. In addition, recruitment was conducted in only two centers in N'Djamena, which may limit the generalizability of the findings to the entire country. Furthermore, only bacteriologically confirmed cases of pulmonary tuberculosis were included, excluding extrapulmonary forms and unconfirmed cases. Finally, drug resistance detection relied solely on the molecular assays Xpert MTB/RIF and Xpert MTB/XDR, without confirmation by phenotypic drug susceptibility testing (DST) or genetic sequencing, which may have affected the estimation of certain resistance patterns.

5. Conclusion

The non-negligible prevalence of multidrug resistance observed in this study confirms that drug-resistant tuberculosis among HIV/TB co-infected patients remains a major public health challenge in Chad. The most notable finding is the identification of one pre-XDR strain among the 3.09% of MDR cases detected in this study, highlighting the importance of rapid molecular diagnosis to improve therapeutic management and limit the spread of multidrug-resistant *Mycobacterium tuberculosis* strains.

Authors' Contributions

The authors contributed to the study design, data collection, and manuscript writing, and declare that they have read and approved the final version of the manuscript.

Acknowledgements

The authors would like to thank the staff of the LNR/PNT, APMS, IRED, and CHU-ATC for their valuable and sincere collaboration.

Conflicts of Interest

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

References

[1] World Health Organization (2025) Addressing the TB/HIV Co-Epidemic: Tuberculosis and HIV Control Strategy. WHO.

[2] Loddenkemper, R., Sagebiel, D. and Brendel, A. (2002) Strategies against Multidrug-Resistant Tuberculosis. *European Respiratory Journal*, **20**, 66s-77s. https://doi.org/10.1183/09031936.02.00401302

[3] World Health Organization (2019) Global Tuberculosis Report 2019: Executive summary. WHO, 1-7. https://doi.org/10.30875/05800ad8-en

[4] Veziris, N. and Robert, J. (2003) Tuberculose multirésistante: Prise en charge. *Lett Infectiol*, **18**, 186-189. https://www.edimark.fr/revues/la-lettre-du-pneumologue/6-novembredecembre-2003-copy-387/tuberculose-multiresistante-prise-en-charge-122003

[5] World Health Organization (2019) Global Tuberculosis Report: Executive Summary. WHO.

[6] World Health Organization Regional Office for Africa (2026) World TB Day 2026—Accelerating Action to End TB in Africa. WHO Regional Office for Africa.

[7] Ossoga, G.W., Ba-Diallo, A., Ngandolo, R., Camara, M., Diop Ndiaye, H., Issifi-Kollo, A., *et al.* (2014) Résistance aux antituberculeux chez les patients atteints de tuberculose pulmonaire dans sept régions du Tchad. *Revue CAMES Santé*, **2**, 18-24.

[8] World Health Organization (2023) Tuberculosis Data. WHO. https://www.who.int/tb/data

[9] Programme National de Lutte contre la Tuberculose (2023) Rapport annuel du Programme national de lutte contre la tuberculose. PNT.

[10] World Health Organization Regional Office for Africa (2026) Tuberculosis (TB): World TB Day 2026—Accelerating Action to End TB in Africa. WHO AFRO.

[11] Ngakoutou, R., Bolti, A.M., Ahmet, A., *et al.* (2022) Évaluation de l'efficacité du traitement an-tituberculeux chez les patients infectés par le VIH au Centre Hospitalier Universi-taire La Référence Nationale de N'Djamena. *JACCR Infectiology*, **4**, 1-7.

[12] Mahamat, A. and Al, E. (2026) Co-Infection VIH/Tuberculose Pulmonaire au Tchad en 2025: Prévalence et résistance à la rifampicine. *Revue Malienne d'Infectiologie et de Microbiologie*, **21**, 71-77. https://doi.org/10.53597/remim.v1i1.3326

[13] Ba Diallo, A., Ossoga, G.W., Daneau, G., Lo, S., Ngandolo, R., Djaibé, C.D., *et al.* (2017) Emergence and Clonal Transmission of Multi-Drug-Resistant Tuberculosis among Patients in Chad. *BMC Infectious Diseases*, **17**, Article No. 579. https://doi.org/10.1186/s12879-017-2671-7

[14] World Health Organization (1998) Laboratory Services in Tuberculosis Control. Part II: Microscopy. WHO.

[15] Kubica, G.P., Dye, W.E., Cohn, M.L. and Middlebrook, G. (1963) Sputum Digestion and Decontamination with N-Acetyl-L-Cysteine-Sodium Hydroxide for Culture of Mycobacteria. *The American Review of Respiratory Disease*, **87**, 775-779.

[16] Boehme, C.C., Nabeta, P., Hillemann, D., Nicol, M.P., Shenai, S., Krapp, F., *et al.* (2010) Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. *New England Journal of Medicine*, **363**, 1005-1015. https://doi.org/10.1056/nejmoa0907847

[17] Helb, D., Jones, M., Story, E., Boehme, C., Wallace, E., Ho, K., *et al.* (2010) Rapid Detection of *Mycobacterium tuberculosis* and Rifampin Resistance by Use of On-Demand, Near-Patient Technology. *Journal of Clinical Microbiology*, **48**, 229-237. https://doi.org/10.1128/jcm.01463-09

[18] Cao, Y., Parmar, H., Gaur, R.L., Lieu, D., Raghunath, S., Via, N., *et al.* (2021) Xpert MTB/XDR: A 10-Color Reflex Assay Suitable for Point-Of-Care Settings to Detect Isoniazid, Fluoroquinolone, and Second-Line-Injectable-Drug Resistance Directly from *Mycobacterium tuberculosis*-Positive Sputum. *Journal of Clinical Microbiology*, **59**, e02314-20. https://doi.org/10.1128/jcm.02314-20

[19] Ismail, N.A., Mvusi, L., Nanoo, A., Dreyer, A., Omar, S.V., Babatunde, S., *et al.* (2018) Prevalence of Drug-Resistant Tuberculosis and Imputed Burden in South Africa: A National and Sub-National Cross-Sectional Survey. *The Lancet Infectious Diseases*,

18, 779-787. https://doi.org/10.1016/s1473-3099(18)30222-6

[20] Nwalozie, R.M., Agbagwa, O.E. and Mac-Fiberesima, G. (2019) A Retrospective Review of Rifampicin-Resistant Mycobacterium Tuberculosis between 2015 and 2017 in Port Harcourt, Nigeria. *International Journal of TROPICAL DISEASE & Health*, **35**, 1-6. https://doi.org/10.9734/ijtdh/2019/v35i230117

[21] Ibrahim, M.M., Isyaka, T.M., Askira, U.M., Umar, J.B., Isa, M.A., Mustapha, A., *et al.* (2022) Trends in the Incidence of Rifampicin Resistant *Mycobacterium tuberculosis* Infection in Northeastern Nigeria. *Scientific African*, **17**, e01341. https://doi.org/10.1016/j.sciaf.2022.e01341

[22] Ogumbo, F., Odero, R., Odhiambo, B., Emojong, P., Okumu, A., Nonoh, J., *et al.* (2022) Isoniazid and Rifampicin Tuberculosis Drug Resistance in HIV Endemic Region of Western Kenya. *East Africa Science*, **4**, 37-47.

[23] Tamunokubie Atemie, K., Ifeoma Udujih, H., Chimezie Nwosu, D., Winners Ndubueze, C. and Stephenson Lawson, D. (2024) Evaluation of Rifampicin Resistance in Tuberculosis Patients Co-Infected with HIV/AIDS in Port-Harcourt, Nigeria. *British Journal of Healthcare and Medical Research*, **11**, 275-289. https://doi.org/10.14738/bjhmr.111.15894

[24] Dinic, L., Akande, P., Idigbe, E.O., Ani, A., Onwujekwe, D., Agbaji, O., *et al.* (2012) Genetic Determinants of Drug-Resistant Tuberculosis among HIV-Infected Patients in Nigeria. *Journal of Clinical Microbiology*, **50**, 2905-2909. https://doi.org/10.1128/jcm.00982-12

[25] Merker, M., Egbe, N.F., Ngangue, Y.R., Vuchas, C., Kohl, T.A., Dreyer, V., *et al.* (2021) Transmission Patterns of Rifampicin Resistant *Mycobacterium tuberculosis* Complex Strains in Cameroon: A Genomic Epidemiological Study. *BMC Infectious Diseases*, **21**, Article No. 891. https://doi.org/10.1186/s12879-021-06593-8

[26] Barnett, B., Gokhale, R.H., Krysiak, R., Kanyemba, C., Chikaonda, T., Bokosi, M., *et al.* (2015) Prevalence of Drug Resistant TB among Outpatients at an HIV/TB Clinic in Lilongwe, Malawi. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, **109**, 763-768. https://doi.org/10.1093/trstmh/trv092

[27] Mamuda, K., Aliyu, M.S., Suleman, M., Ibrahim, A.K., Song, A.M., Garba, B.Y., *et al.* (2026) Frequency, Genetic Mechanisms and Factors Associated with Fluoroquinolone (pre-XDR) and Aminoglycoside Resistance among Multidrug-Resistant Tuberculosis Cases in Northwestern Nigeria. *BMC Infectious Diseases*, **26**, Article No. 910. https://doi.org/10.1186/s12879-026-13191-z

[28] Nehru, V.J., Jose Vandakunnel, M., Brammacharry, U., Ramachandra, V., Pradhabane, G., Mani, B.R., *et al.* (2024) Risk Assessment and Transmission of Fluoroquinolone Resistance in Drug-Resistant Pulmonary Tuberculosis: A Retrospective Genomic Epidemiology Study. *Scientific Reports*, **14**, Article No. 19719. https://doi.org/10.1038/s41598-024-70535-y

[29] Che, Y., Lu, Y., Zhu, Y., He, T., Li, X., Gao, J., *et al.* (2024) Surveillance of Fluoroquinolones Resistance in Rifampicin-Susceptible Tuberculosis in Eastern China with Whole-Genome Sequencing-Based Approach. *Frontiers in Microbiology*, **15**, Article 1413618. https://doi.org/10.3389/fmicb.2024.1413618

[30] World Health Organization (2019) WHO Consolidated Guidelines on Drug-Resistant Tuberculosis Treatment. WHO.

[31] Lee, C.M., Lee, E., Bang, J.H., Park, S., Park, W.B., Oh, M., *et al.* (2021) Prevalence of Multidrug-Resistant Tuberculosis in HIV/Tuberculosis Co-Infected Patients. *Infection & Chemotherapy*, **53**, 792-795. https://doi.org/10.3947/ic.2021.0085

[32] Ouedraogo, S.M., Ouédraogo, A.R., Birba, E., *et al.* (2014) Tuberculose multirésis-

tante à Bobo-Dioulasso: Aspects épidémiologiques, cliniques, radiographiques et évolutifs. *RAMFI*, **1**, 30-34.

[33] Weyer, K., Brand, J., Lancaster, J., Levin, J. and Vanderwalt, M. (2007) Determinants of Multi-Drug-Resistant Tuberculosis in South Africa: Results from a National Survey. *South African Medical Journal*, **97**, 1120-1128.

[34] Doungous, D.M., Togde, G.L.D., Chatté, A., Oumar, O., Soré, S., Djimenan, B., *et al.* (2026) Biological Monitoring of Patients under First-Line Antituberculous Treatment in Chad from 2021 to 2022. *Journal of Tuberculosis Research*, **14**, 18-27. https://doi.org/10.4236/jtr.2026.141003

[35] Seid, A., Girma, Y., Abebe, A., Dereb, E., Kassa, M. and Berhane, N. (2023) Characteristics of TB/HIV Co-Infection and Patterns of Multidrug-Resistance Tuberculosis in the Northwest Amhara, Ethiopia. *Infection and Drug Resistance*, **16**, 3829-3845. https://doi.org/10.2147/idr.s412951

[36] Dlinga, D., Ngakoutou, R., Ahmet, A., Moussa, A.M.M., Adjougoulta, K.A., Adjougoulta, K.D.B., *et al.* (2023) Evaluation of the Profiles of Patients with Multidrug Resistant Tuberculosis at the University Hospital Center La Référence Nationale de N'Djamena-Chad. *Journal de la Fonction Ventilatoire et de Pneumologie*, **43**, 1-64.

[37] Hirpa, S., Medhin, G., Girma, B., Melese, M., Mekonen, A., Suarez, P., *et al.* (2013) Determinants of Multidrug-Resistant Tuberculosis in Patients Who Underwent First-Line Treatment in Addis Ababa: A Case Control Study. *BMC Public Health*, **13**, Article No. 782. https://doi.org/10.1186/1471-2458-13-782

[38] Misombo-Kalabela, A., Nguefack-Tsague, G., Mireille, G.C., Ze, E.A., Diangs, K., Panda, T., *et al.* (2016) Facteurs de risque de la tuberculose multi-résistante dans la ville de Kinshasa en République Démocratique du Congo. *Pan African Medical Journal*, **23**, Article 157. https://doi.org/10.11604/pamj.2016.23.157.6137

[39] Kombila, U.D., Boulingui, C.M., Ibinga, L.D., Mounguengui, D., N'gomanda, F., Massolou, R., *et al.* (2021) Difficultés et obstacles dans la prise en charge de la tuberculose multirésistante au Centre Hospitalier Universitaire de Libreville: étude rétrospective de 2017 à 2020. *Health Sciences and Disease*, **22**, 46-50.

[40] Okemba-Okombi, F.H., Ndinga Essango, E., Kaswa Kayomo, M., Ossale Abacka, B.K., Bopaka, R.G. and Atipo Ibara, B.I. (2020) Multi-Resistant Tuberculosis in Brazzaville: Epidemiological, Clinical, Radiographic and Progressive Aspects. *Journal of Functional Ventilation and Pulmonology*, **33**, 1-6.

[41] Desissa, F., Workineh, T. and Beyene, T. (2018) Risk Factors for the Occurrence of Multidrug-Resistant Tuberculosis among Patients Undergoing Multidrug-Resistant Tuberculosis Treatment in East Shoa, Ethiopia. *BMC Public Health*, **18**, Article No. 422. https://doi.org/10.1186/s12889-018-5371-3

[42] LaFreniere, M., Dam, D., Strudwick, L. and McDermott, S. (2020) Résistance aux antituberculeux au Canada: 2018. *Relevé des maladies transmissibles au Canada*, **46**, 10-16. https://doi.org/10.14745/ccdr.v46i01a02f

[43] Fiogbé, A.A., Adjoh, K.S., Ouedraogo, A.R,. Maïga, A.I., Wateba, M.I., Okemba-Okombi, F.H., *et al.* (2014) Co-Infection VIH/Tuberculose en milieu rural au Bénin: Cas de la zone sanitaire Djougou-Ouaké-Copargo. *Mali Medical*, **29**, 15-22.

[44] Brust, J.C.M., Shah, N.S., Mlisana, K., Moodley, P., Allana, S., Campbell, A., *et al.* (2017) Improved Survival and Cure Rates with Concurrent Treatment for Multidrug-Resistant Tuberculosis-Human Immunodeficiency Virus Coinfection in South Africa. *Clinical Infectious Diseases*, **66**, 1246-1253. https://doi.org/10.1093/cid/cix1125