

Research Article

Genotypic Characterization of *Mycobacterium Tuberculosis* Strains Resistant to Rifampicin, Isoniazid and Second-Line Antibiotics in Chad

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Citation: Boilengar D, et al. Genotypic Characterization of *Mycobacterium Tuberculosis* Strains Resistant to Rifampicin, Isoniazid and Second-Line Antibiotics in Chad. J Clin Immunol Microbiol. 2024;5(1):1-14.

<http://dx.doi.org/10.46889/JCIM.2024.5108>

Received Date: 15-03-2024

Accepted Date: 02-04-2024

Published Date: 09-04-2024



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Abstract

Monitoring drug resistance and identifying the genetic basis of resistance to first- and second-line anti-tuberculosis (anti-TB) drugs provides important information to optimize patient care. The present study was an observational, cross-sectional and analytical study aimed at determining the prevalence of resistance genes to rifampicin, isoniazid and resistant and multi-resistant genes with mutations associated with second-line anti-tuberculosis drugs (Levofloxacin Amikacin, Kanamycin and Capreomycin) among strains of *Mycobacterium tuberculosis* in nine hospitals in four provinces of Chad with three new molecular techniques. The TB-LAMP molecular technique made it possible to detect 264 (25.14%) strains of *Mycobacterium tuberculosis* complex among 1050 people referred for tuberculosis research, including 37 cases in relapse and 237 new cases under treatment or not without clinical improvement. The average age of the patients was 34.15 years with the extremes ranging from 12 to 77 years. The sex ratio was 3.33 in favor of men. The GeneXpert made it possible to confirm the 264 positive cases of *Mycobacterium tuberculosis* (MTB+) of which 76% of men and 24% of women had the MTB+ profile sensitive to rifampicin, and 80% of men and 20% of women harbored the Mycobacterium complex. rifampicin-resistant tuberculosis. The determination of resistance genes including 13% (rpoB) to rifampicin, 4% (inhA) to isoniazid and 8% (rpoB/KatG and/or KatG/inhA) multi-resistant (MDR) genes was carried out using the Line Probe Assay technique. This technique also made it possible to determine resistance genes with associated mutations including 13.6% (gyrA and gyrB), 7.6% (eis), 6.1% (rrs and eis) and 10.6% being mutations affecting the different alleles on the chromosomes of the *Mycobacterium tuberculosis* complex.

This study highlighted the emergence of resistance to rifampicin and isoniazid as well as resistance with mutations associated with second-line anti-tuberculosis drugs. It raises the

need to implement an effective surveillance system to detect the resistance of *Mycobacterium tuberculosis* to anti-tuberculosis drugs in Chad and even in Central Africa.

Keywords: *Mycobacterium tuberculosis*; Resistance; Anti-Tuberculosis Drugs; Molecular Technique; Chad

Introduction

Tuberculosis is an infectious and contagious disease caused by a mycobacterium of the *Mycobacterium tuberculosis* Complex (MTB) [1]. The emergence of resistance to anti-tuberculosis drugs in many countries has become a public health problem and

constitutes an obstacle to effective control of tuberculosis [2]. The burden of drug-resistant tuberculosis increases by 3% in 2020-2021 and is equivalent to 450,000 new cases of rifampicin resistance [3]. According to the WHO, in Chad, tuberculosis is very widespread, the incidence rate is 1.41% or 144/100,000 people [4]. The high incidence of TB and drug-resistant TB in vulnerable populations highlights the problem and factors associated with TB [5]. The emergence of drug resistance in *Mycobacterium tuberculosis* is due to non-compliance and misuse of anti-TB drugs without laboratory evidence which has contributed immensely to genetic mutations, which subsequently lead to drug resistance. frequently prescribed anti-tuberculosis drugs [6, 7].

In Chad, a previous study on the drug resistance of tuberculosis in sputum in 232 new patients followed in three hospitals reported 58% (n=135) patients detected positive for tuberculosis, of whom 3 (2.2%) presented multidrug resistance to tuberculosis (MDR-TB). The rate of resistance to at least one anti-tuberculosis antibiotic was 20% [8]. Tuberculosis strains are resistant to at least two of the most potent anti-tuberculosis drugs: isoniazid and rifampicin [9,10]. Thus, to combat tuberculosis, Chad's national tuberculosis control program (PNT) has focused on early diagnosis and prevention of tuberculosis among patients with rifampicin-resistant tuberculosis (RR-TB)) [11,12]. It is suggested that if one or more TB-related signs or symptoms are present, the GeneXpert test should be routinely performed for confirmation of active TB cases among household contacts of MDR-TB, before starting TB treatment [13].

The objective of this study was to determine the frequency and genetic characteristics of resistance to rifampicin and isoniazid as well as second-line anti-tuberculosis drugs of *Mycobacterium tuberculosis* species isolated from patients with tuberculosis in six health facilities in N' Djamena and three provinces in Chad using molecular techniques (TB-Lamp, GeneXpert and Line Probe Assay) to better manage tuberculosis patients in Chad.

Material and Methods

Framework of the Study and Conduct of The Research Work

This is an observational, cross-sectional and analytical study, carried out in six hospitals in N'Djamena and three in the provinces for the recruitment of people affected by tuberculosis in Chad.

Sputum samples were collected from October 1, 2022 to March 31, 2023 from tuberculosis patients and people with signs suggestive of tuberculosis for the search for *Mycobacterium tuberculosis* in six hospitals in the province of N'Djamena (Reference University Hospital Center of N'Djamena (CHU-RN) ; University Hospital Center for Mother and Child (CHU-ME); Renaissance University Hospital Center (CHU-R); Military Instruction Hospital (HMI); Chad-China Friendship Hospital (HATC) and the Good Samaritan University Hospital Center (CHU-BS)) and three provincial hospitals (Chari Baguirmi, Hadjar Lamis, Mayo Kebbi Ouest) and others from Kousseri in Cameroon were tested:

- At the Laboratory Mycobacteria Unit of the National Reference University Hospital Center (CHU-RN) of N'Djamena
- At the National Reference Laboratory of the National Tuberculosis Control Program (LNR-PNT) of Chad

A total of 264 non-duplicate *Mycobacterium tuberculosis* isolates were collected from 1050 samples screened for resistance genes (rpoB, katG, gyrA, gyrB, rrs, eis and inhA) and associated mutations. Patient volunteers (each of whom signed an informed consent form) were recruited by convenience sampling at different stages of treatment with the aim of searching for resistant strains with mutant genes of *Mycobacterium tuberculosis*. The study included 27 relapsed patients and 237 new cases under treatment or not without clinical improvement. Bacterial DNA was extracted from sputum using the GenoLyse kit (Hain Life science), according to the manufacturer's protocol. Extracted DNA was stored at -20°C until processing.

Detection of Tuberculosis by Three Molecular techniques in Chad [14-18]

Detection of Tuberculosis by the TB-LAMP Automaton

TB-LAMP is a molecular diagnostic technique that has enabled sensitive and accurate detection of tuberculosis. It is easy to use and gives reliable results within an hour. The process takes place in four 4 stages, namely:

- Sputum preparation: 60µL of sputum was transferred to the heating tube, shaken to mix well and incubated in the HumaLoop T heating unit at 90°C for 5 minutes
- DNA extraction: The sample pretreated in the heating tube (lysed) was transferred to the absorbent tube with absorbent

powder which removes all possible inhibitors of the LAMP reaction. The purified DNA was extracted and directly transferred to the LAMP reaction tube

- Amplification: DNA transferred to the LAMP reaction tube was incubated for 2 minutes at room temperature to replenish the reagents in the cap, then homogenized several times and tapped until the reaction mixture accumulated at the bottom of the tube. The reaction tube was then transferred to the HumaLoop T reaction unit at 67 °C for 45 minutes

Reading the Results

The reaction tube was finally inserted into the detection unit of the UV lamp and the lamp was turned on:

- A positive result gives a green light
- A negative result does not give fluorescence

Real-time turbidity reading and automatic results reporting

Detection of Rifampicin Sensitivity and Resistance Genes with The Genexpert Automated System

Principle: it is based on amplification of a fragment of the *rpoB* gene containing the central region at 81 base pairs and fragments of the target sequences of the IS1081 and IS6110 insertion elements with multiple copies by primers.

Using a Pasteur pipette, 2 mL of sputum and 4 mL of the reagent were taken and mixed in another sterile jar. The mixture was vortexed and then incubated at room temperature for 10 minutes. The pot was vortexed again and incubated at room temperature for 5 minutes. Then, 2 mL of the liquefied mixture was aspirated and transferred into the Xpert® MTB/RIF ULTRA cartridge and the test was run for 1 hour 30 minutes.

Detection of Rifampicin and Isoniazid Resistance Genes Using the Line Probe Assay (Hain)

Principle: it is based on DNA-STRIP technology and includes DNA extraction, master mix preparation, multiplex amplification with biotinylated primers and detection by reverse hybridization.

Sputum samples were decontaminated by the BD BBL™ MycoPrep kit containing N-Acetyl L-Cysteine-sodium hydroxide and phosphate buffer. DNA was extracted from the samples using the GenoLyse kit (Hain Life Science). 500 µL of the decontaminated sample was taken and transferred to the Ependorf tubes and centrifuged at 10,000 rpm for 15 minutes. After centrifugation, only the pellet was kept to which 100 µL of lysis buffer solution (A-LYS) was added, mixed by vortexing and incubated for 5 minutes at 95 °C in a water bath. Afterwards we added 100 µL of neutralization buffer (A-NB), then vortexed the mixture for 5 seconds and centrifuged quickly for 5 minutes in order to bring the DNA from the pellet to the supernatant. Our interest was focused on the supernatant. Then the extracted DNA was amplified by PCR and to do this, we had to prepare 45 µL of mixture (10 µL of MixA + 35 µL of MixB) for each sample, then we introduced 45 µL of mixture (mixA+ mixB) in each tube. Then 5 µL of the DNA from each sample was added. After amplification, the PCR products (amplicons) were hybridized with the probes fixed on the strip. To do this, 20 µL of denaturation solution (DEN, blue) were distributed in the corner of each of the wells used to which 20 µL of amplified DNA were added then the mixture was homogenized and incubated at room temperature for 5 minutes. 1 mL of preheated hybridization buffer was added to each well and then gently shaken the tray until the solution had a homogeneous color. Subsequently, a strip was placed in each well. We placed the tray in the shaking water bath/TwinCubator and incubated for 30 minutes at 45°C. We then aspirated the hybridization buffer completely then added 1 mL of stringent wash solution to each strip and incubated for 15 minutes at 45°C in a shaking water bath/TwinCubator. Then, the stringent wash solution was completely vacuumed up. Each strip was washed once with 1 mL of rinse solution for 1 minute on the shaking platform/TwinCubator (pour out the RIN after incubation) then added 1 mL of diluted conjugate to each strip and incubated for 30 minutes on the shaking platform/TwinCubator. The solution was removed, washed each strip twice for 1 minute with 1 mL of rinsing solution and once for 1 minute with 1 mL of distilled water on a shaking platform/TwinCubator (pour the solution into each time) and added 1 mL of diluted substrate to each well then incubated away from light for 5 min, rinsed briefly twice with distilled water and dried the strips.

Line Probe Assay (LPA) or reverse hybridization strip tests are DNA strip tests which make it possible to determine the drug resistance profile of a strain of the *Mycobacterium tuberculosis* Complex through the pattern of amplicon binding (amplification products DNA) to probes targeting the mutations most often associated with resistance of the *Mycobacterium tuberculosis* Complex

to first- and second-line anti-tuberculosis drugs and to probes targeting the corresponding wild-type DNA sequence.

Written and Signed Informed Consent

Sir/Madam,

We would like to take a sample of your sputum, this is a procedure usually done to look for the microorganisms responsible for pulmonary tuberculosis.

The sample will not cause any risk to your health, it will be used to identify the pathogen(s) responsible for your health problem. Under no circumstances will other examinations be carried out without your consent. The results obtained will be made available to you and will undoubtedly provide better insight into the cause of your illness.

You will be informed of any change in the purpose of the research on the samples and you will be able to object.

Sir/Madam, your participation is essential for the completion of this study which will allow us to contribute to improving your care.

Patient signature and telephone number

Data Analysis

Microsoft office Word and Microsoft office Excel were used to enter and analyze the results. The chi-square test was used to study the correlations between variables at a margin of error of 5%.

Results

Mapping of the Localities of N'Djamena and Provinces Surveyed in Chad

Figure 1 illustrates the different health facilities in the provinces where the surveys took place (Chari Baguirmi, Hadjar Lamis, Mayo Kebbi Ouest and N'Djamena). Chad is located between 7th and 24th degrees north latitude and 13th and 24th degrees east longitude. It covers an area of 1,284,000 km²; it is the fifth largest country in Africa after Sudan, Algeria, Zaire and Libya. From North to South, it extends over 1,700 km and, from East to West, over 1,000 km. It shares its borders with Libya to the north, Sudan to the east, the Central African Republic to the south and Cameroon, Nigeria and Niger to the west. Mayo Kebbi province is located southwest of N'Djamena and shares its borders with Cameroon.

The province of Hadjar Lamis is located 300 km north of N'Djamena. The province of Chari Baguirmi is contiguous to the South and South-West with the province of N'Djamena. The country belongs politically and economically to Central Africa, but due to the similarities in climatic conditions, it is also linked to the Sahelian countries. The geolocation of Chad would certainly have contributed to the transmission of new cases and cases of relapses of tuberculosis.

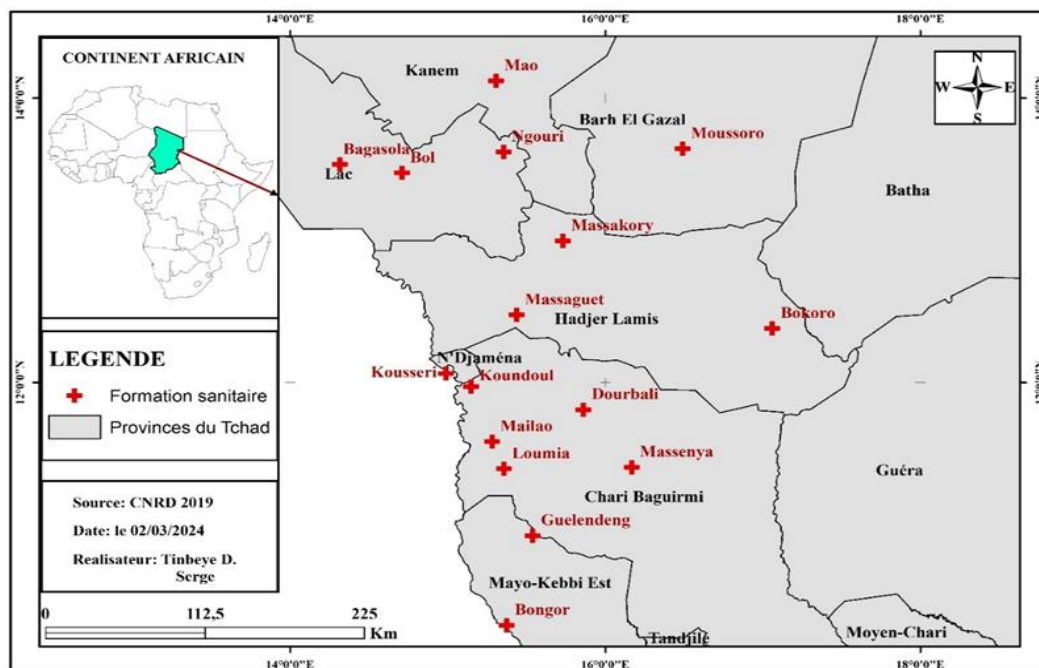


Figure 1: Mapping of the localities surveyed.

Overall Prevalence of Tuberculosis

The study was carried out on 1050 people referred for research into pulmonary tuberculosis. Among the 1050 samples tested by the TB-LAMP molecular technique, 264 (25.14%) were positive for *Mycobacterium tuberculosis* complex (MTB+) and 786 (75%) negatives ($p = 0.01$: significant difference in favor of negative tests).

Distribution of Positive Cases by Gender

The male gender represented 77% with a number of 203 and the female gender accounted for 61 or 23.11% ($p = 0.001$: significant difference in favor of the male gender). The sex ratio was 3.33 in favor of men. The average age of the patients was 34.15 years with the extremes ranging from 12 to 77 years.

Distribution of Patients According to Origin

Fig. 2 illustrates the distribution of patients according to health facilities. CHU-RN patients were mainly represented followed by HMI and provincial towns with the proportions of 66%, 14% and 9% respectively.

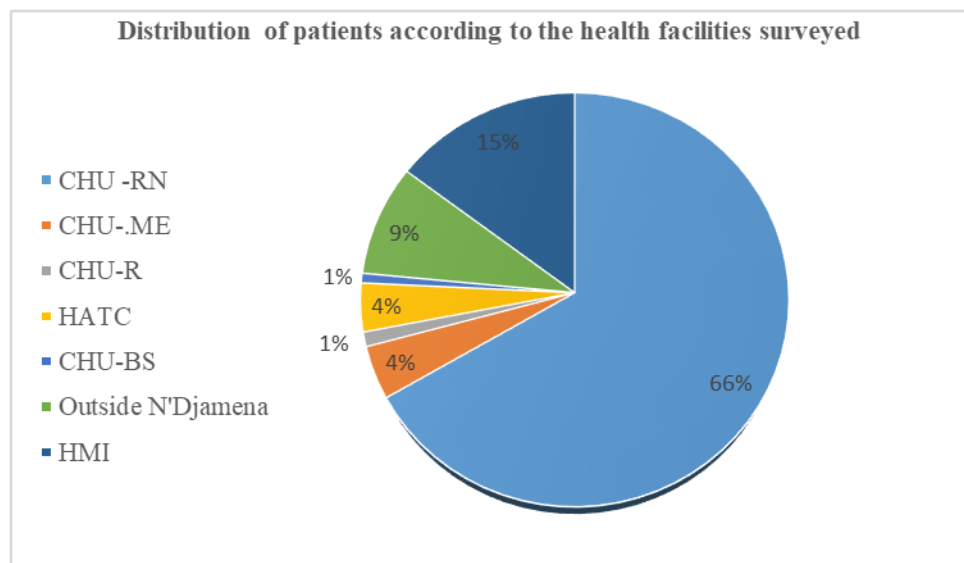


Figure 2: Distribution of patients according to health facilities. CHU-RN: National Reference University Hospital Center; CHU-ME: University Hospital Center for Mother and Child; CHU-R: Renaissance University Hospital Center; HATC: Chad-China Friendship Hospital; CHU-BS: Good Samaritan University Hospital Center; Outside N'Djamena: the towns of the provinces of Chari Baguirmi, Hadjar Lamis, Mayo Kebbi West; HMI: Military Instruction Hospital.

Distribution of Patients According to Profession

Fig. 3 illustrates the distribution of patients by profession. Soldiers and housewives were in the lead (18.2%) each followed by the unemployed (15.5%) and traders (14%).

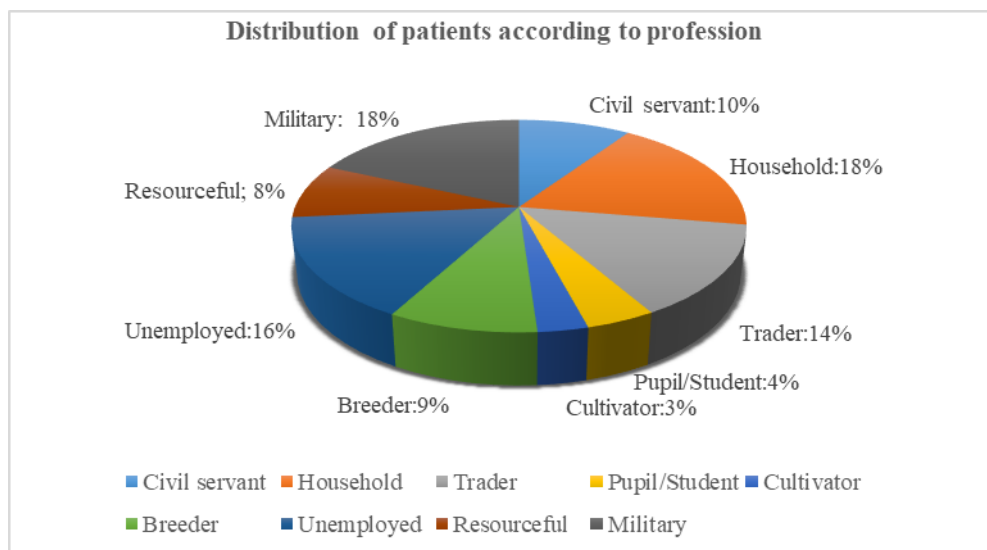


Figure 3: Distribution of patients according to profession.

Distribution of Patients According to Level of Education

Fig. 4 illustrates the distribution of patients according to educational level. Those not in school come first, followed by primary, secondary and higher education with the proportions of 121 (46%), 77 (29%), 44 (17%) and 22 (8%) respectively.

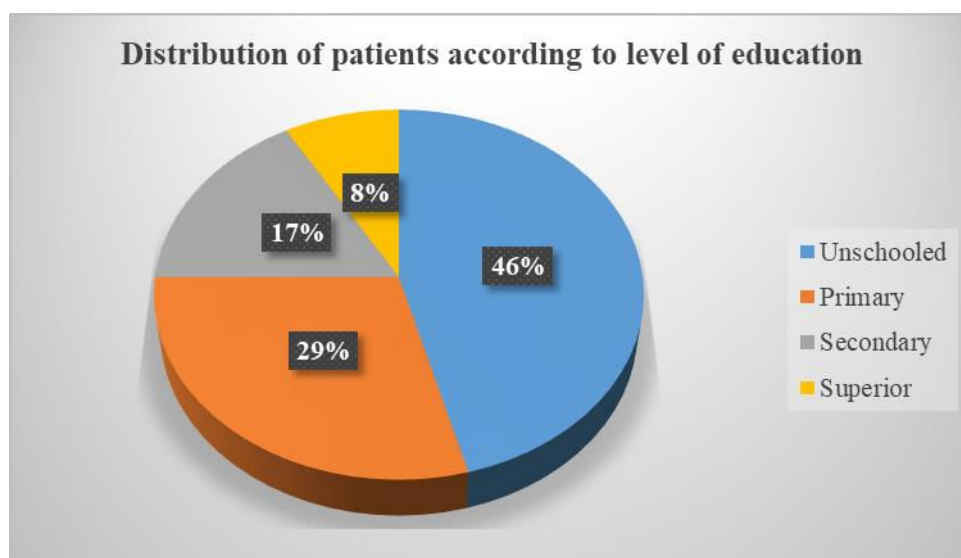


Figure 4: Distribution of patients according to level of education.

Susceptibility and Resistance Profile of Mycobacterium tuberculosis to First-Line Anti-Tuberculosis Drugs

Of the 264 patients detected *Mycobacterium tuberculosis* positive (MTB+), 198 (75%) had the MTB+ profile sensitive to anti-tuberculosis drugs and 66 (25%) were resistant to first-line anti-tuberculosis drugs. Of the 66 MTB+ resistant, 34 (13%) were resistant to rifampicin, 10 (4%) were resistant to isoniazid and 22 (8%) were cases of multidrug resistance (Fig. 5).

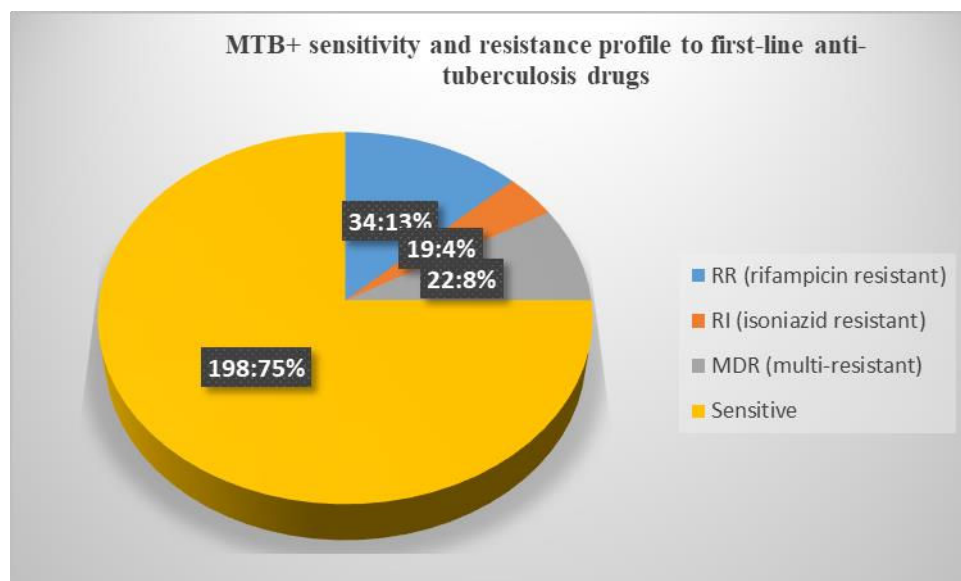


Figure 5: MTB+ sensitivity and resistance profile to first-line anti-tuberculosis drugs. (MTB+, RIF+) = *Mycobacterium tuberculosis* sensitive to rifampicin; (MTB+, RIF-) = *Mycobacterium tuberculosis* resistant to rifampicin.

Distribution of Patients According to The Profile of Sensitivity and Resistance to Rifampicin According to Sex

Fig. 6 illustrates the distribution of patients with the MTB+ profile sensitive and resistant to rifampicin. Of the 264 MTB+, 76% of men (n=150) and 24% (n=48) of women had the MTB+ profile sensitive to rifampicin. 80% (n=53) of men and 20% (n=13) of women harbored rifampin-resistant *Mycobacterium tuberculosis* complex.

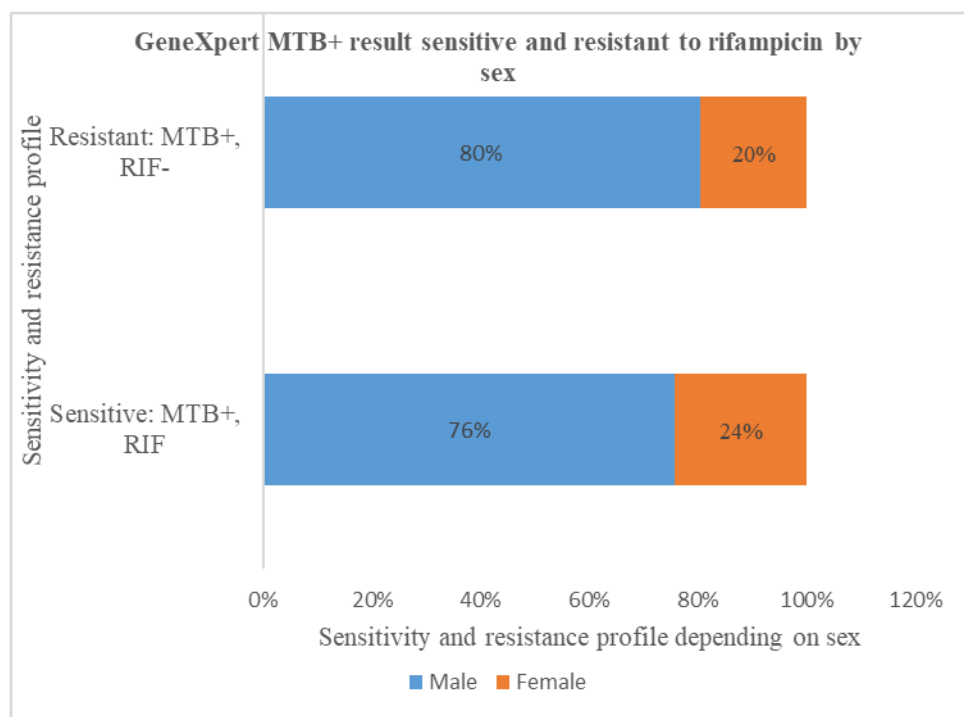


Figure 6: Distribution of patients according to the profile of sensitivity and resistance to rifampicin according to sex.

Distribution of Resistance and Sensitivity Genes to First-Line Anti-Tuberculosis Drugs

Of the 264 positive cases (MTB+), 75% (n=198) were sensitive and 25% (n=66) were resistant to first-line anti-tuberculosis drugs. Among the 25% of MTB+, the Line Probe Assay (Hain) detected 13% of genes (rpoB) resistant to rifampicin, 4% (inhA) resistant to isoniazid and 8% (rpoB/KatG and/or KatG/ inhA) were Multidrug Resistance (MDR) genes (Fig. 7).

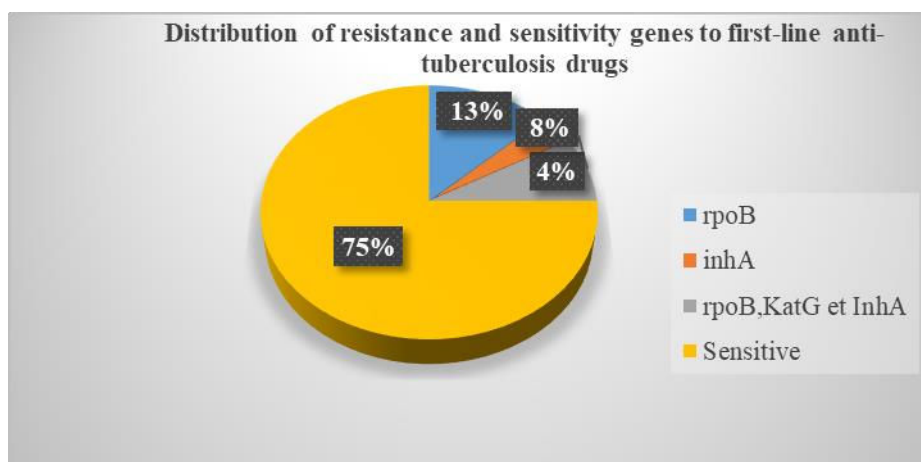


Figure 7: Distribution of resistance and sensitivity genes to first-line anti-tuberculosis.

Distribution of Resistance Genes with Mutations Associated with Second-Line Anti-Tuberculosis Drugs

The results of resistance associated with mutations were presented as follows: of the 25% (n=66) of MTB+ harboring resistant and multi-resistant genes, 62.1% did not present any mutation associated with second-line anti-tuberculosis drugs. Among the genes detected with mutations associated with antibiotics (Levofloxacin (Lfx), Amikacin (Am), Kanamycin (Km) and Capreomycin (Cm)) by the Line Probe Assay technique, 13.6% (gyrA and gyrB) were due to gyrase enzymes, 7.6% (eis) and 6.1% (rrs and eis) and 10.6% being mutations affecting the different alleles at the level of the chromosomes of the *Mycobacterium tuberculosis* complex.

The mechanism of resistance to Levofloxacin was manifested by point mutations that occurred in specific regions of two enzymes: DNA gyrase A, DNA gyrase B. Mutations in the quinolone resistance determinants of regions (QRDRs) of gyrase A and B at the locus of these genes are amplified by PCR and sequenced. The DNA sequences were translated into protein sequences. The sequences of these strains were compared to those of certain strains sensitive to Ciprofloxacin in order to look for amino acid substitutions conferring resistance (Fig. 8).

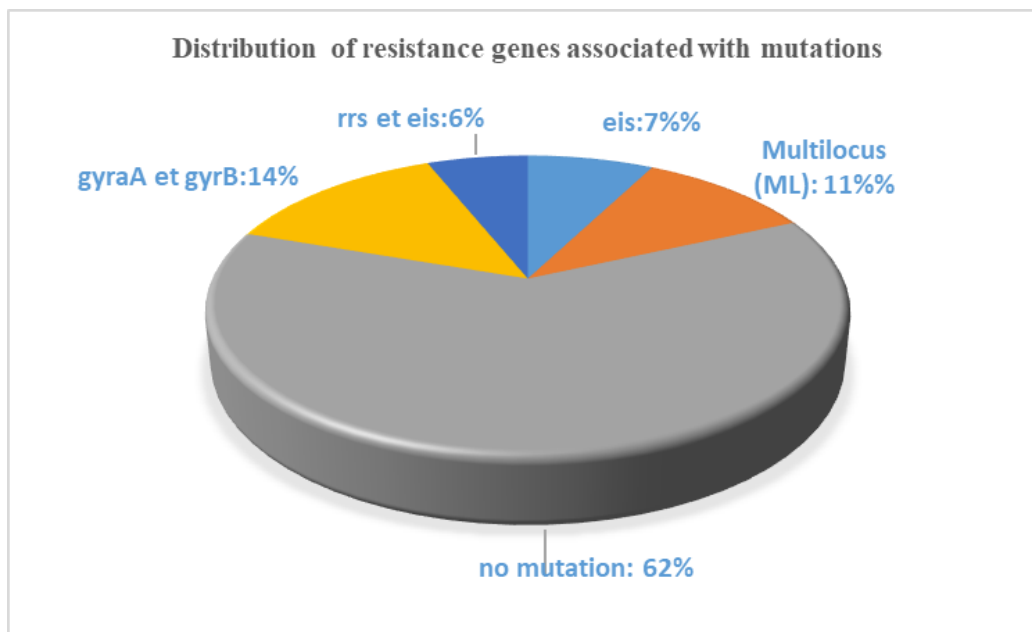


Figure 8: Distribution of resistance genes associated with mutations.

Distribution of Genes According to the Types of Resistance and Mutations Caused by MTB+ Strains

Among rifampin-resistant isolates, the majority (61%) of mutations occurred at codon H526D of the rpoB gene, followed by 23% of genes (katG) with mutations at codons S315T, 23% of isolates had mutations at the S315T codon of the katG gene and 11% (inhA) of isoniazid-resistant genes had mutations at the C15T codon. Among the 4 cases of multi-resistance, the majority (14%) of mutations were observed at the S315T codon of the katG gene, followed by mutations at the H526D (9.1%) and H516D (6.1%) codons of the katG gene. rpoB and C15T genes (4.5%) of the inhA gene. Two cases of resistance to rifampicin were observed in patients co-infected with tuberculosis and Hepatitis B with mutations that occurred at the H526D codon of the rpoB gene (Table 1,2).

Types of Resistance	Gene	Target sequence	Mutated codon	Effective	%	Result			
Rifampicin resistance	rpoB	MUT2B	H526D	24	36,4				RR
	rpoB	MUT3	H526D	10	15,1				RR
Isoniazid resistance	InhA	MUT1	C15T	4	6,1				RI
	katG	MUT1	S315T	6	9,1				RI
Multi-resistance	katG	MUT1	S315T	9	14	KatG/rpoB	KatG/ RpoB/ inhA	MDR	MDR
	rpoB	MUT2B	H526D	6	9,1				
	rpoB	MUT1	H516D	4	6,1				
	inhA	MUT1	C15T	3	4,5				
Total				66	100,00				

RR= Resistance to Rifampicin; RI= Isoniazid Resistance; MDR=Multi-Resistance; % = Percentage

Table 1: Summary of types of resistance and mutations conferring resistance to rifampicin and/or isoniazid.

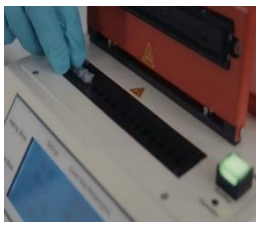
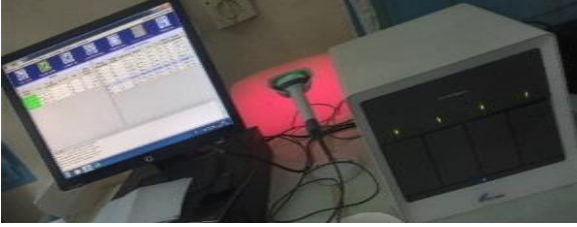
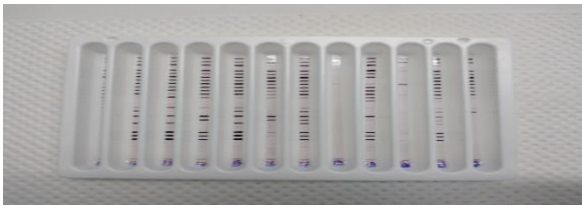
1	a: TB-LAMP: HumaLoop T b: sputum sample c: transfer and lysis of the sputum sample			
2	d: DNA extraction e: amplification f: reading TB-LAMP results (appearance: milky (positive), clear (negative))			
3	g: Gene Xpert h: preparation of sputum under the safety hood i: prepared sputum transfer into GeneXpert cartridge			
4	j: introduction of the cartridge into the GeneXpert automaton k: reading the results on the GeneXpert automaton screen			
5	l: sample preparation for the Line Probe Assay technique m: DNA extraction n: amplification			
6	o: hybridization and migration of genes on strip Genotype MTBDR+ (version 2.0; 48STRIP) incubated on Twincubator p: reading the results			

Table 2: The biotechnological steps for the detection of *Mycobacterium tuberculosis* and the *rpoB*, *katG*, *gyrA*, *gyrB*, *rrs*, *eis* and *inhA* genes by three molecular techniques.

Discussion

The WHO has stated that treatment with first-line anti-tuberculosis drugs (rifampicin and isoniazid) can effectively control the incidence of tuberculosis and contribute to the elimination of the burden of tuberculosis worldwide, including Chad [2,19]. The emergence of drug resistance in tuberculosis is a major concern for the implementation of disease intervention programs [20]. No previous study has noted the presence of resistance genes associated with mutations at different loci of the *Mycobacterium tuberculosis* complex in Chad. This study made it possible to analyze cases of drug-resistant tuberculosis by detecting the sites making it possible to determine the *rpoB*, *katG*, *gyrA*, *gyrB*, *rrs*, *eis* and *inhA* genes potentially associated with resistance to both first and second anti-tuberculosis drugs. online, using samples from 264 patients with tuberculosis from 2022 to 2023. Similar studies were reported in China in 2023 [21,22]. During this research, a total of 1050 sputum samples were meticulously collected and subjected to thorough examination using TB-LAMP, GeneXpert and Line Probe Assay diagnostic tests (Table 2). It was discovered that among the aforementioned samples, 264 tested positive for the presence of tuberculosis, which translates into a frequency of 25% (264/1050) including 27 patients in relapse and 237 new cases under treatment or not without clinical improvement. This clearly highlights the fact that tuberculosis continues to persist within the geographical limits of Chad. The frequency observed in this study is comparable to the results of Kwaghe, et al., in Nigeria, where they reported a frequency of 22.1% [23]. Furthermore, it is slightly lower than the frequency reported by Gashaw, et al., in Ethiopia, which was 29.2%, but higher than that observed by Arega, et al., in Ethiopia, who reported the frequency of 15.11% [24,25]. This divergence in frequency of occurrence could be explained by societal customs, migration patterns and interactions between different populations, and in particular by HIV prevalence.

In the present investigation, a total of 203 males (76%) sample and 61 females (24%) with pulmonary tuberculosis were identified. The predominance of men in this regard has already been documented elsewhere, and these results are superimposable to the data presented by the WHO, in which it is argued that men are more susceptible to the disease than women [10,26,27].

Concerning the level of education and profession, this study noted 46% of unschooled patients, soldiers and housewives (18.2%) each followed by the unemployed (15.5%) and 14% of traders. The high prevalence of Tuberculosis (TB) among men could be attributed to their exposure to various risk factors, such as smoking, drug abuse, occupational respiratory problems, poverty, malnutrition and some factors associated with immunosuppression [2].

Regarding sensitivity and resistance testing, the present study determined the resistance profile and sensitivity of the *Mycobacterium tuberculosis* complex to the main first- and second-line drugs used, namely rifampicin, isoniazid and antibiotics (Levofloxacin, Amikacin, Kanamycin and Capreomycin) by GeneXpert and Line Probe Assay techniques (Table 2). The overall resistance rate observed in this study was 25% and the susceptibility rate was 75%, indicating the presence of resistance to at least one anti-tuberculosis drug. The results of this investigation are similar to those of a previous study conducted in Chad by Awa Ba Diallo, et al., in which they documented a resistance rate of 23.4% [9]. This rate exceeds the results obtained by Gashaw, et al., in 2021 in Ethiopia, where they reported a frequency of 16.8% [24]. It would be essential to recognize the variations in resistance rates between different geographic regions and the potential factors that contributed to these disparities. Potential reasons for variations in drug resistance rates could be elucidated by disparities in the sample sizes used, the locations where studies were conducted, as well as the individuals included in the samples. The frequency of multi-resistance was 34.37% in this series, which is significantly higher than the rate of 3.07% reported in a previous survey conducted in Chad in 2015 [4]. This gap highlights the dissemination of resistant strains within the population through the increasing misuse of anti-tuberculosis drugs, a trend that continues to increase. The frequency of this particular event bears a striking resemblance to the data obtained in Cameroon, as reported by Tiani, et al., where a rate of 4.5% was observed [26]. Furthermore, it should be noted that the aforementioned frequency is slightly lower than the results of an extensive survey conducted in Ethiopia, as presented by Arega, et al., who reported a prevalence rate of 5, 64% [25]. The reason for this relatively low incidence of multidrug-resistant strains could undoubtedly be attributed to the fact that all patients included in this study were individuals who had recently received their initial diagnosis and, therefore, had never experienced any form of therapeutic intervention, which would correspond to the concept of primary resistance. The prevalence of resistance to rifampicin and isoniazid in this series was 13% and 4% respectively. These rates are comparable to numerous studies carried out in Chad and elsewhere [4,27-29]. It should be noted, however, that these current frequencies are not consistent with a number of studies that highlight the importance of rifampicin monoresistance compared to isoniazid monoresistance, albeit in varying proportions. Other studies also propose the opposite

idea, suggesting that isoniazid monoresistance may be of greater importance. This divergence in results was illustrated by the work of Gashaw, et al., in 2021 [24].

In addition, this study was able to determine by the Line Probe Assay technique (Table 2) the resistance genes with mutations associated with second-line anti-tuberculosis drugs: (Levofloxacin, Amikacin, Kanamycin and Capreomycin (Table 1). Extensively resistant tuberculosis (UR) to a fluoroquinolone and at least one of the three second-line injectable drugs (capreomycin, kanamycin and amikacin) was also reported by WHO experts in 2021 [2].

Compared to rifampin-resistant isolates associated with mutations, the majority (61%) of mutations occurred at the H526D codon of the *rpoB* gene, followed by 23% of genes (*katG*) with mutations at the S315T codons. among 27 samples of relapsed cases were identified. Rifampicin-resistant tuberculosis (RR-TB) in relapsed patients has been reported at a rate half (12%) of that in this study series by Elion Assiana, et al., in the Republic of Congo [20]. Tuberculosis resistant to isoniazid (Hr tuberculosis) and sensitive to rifampicin was observed in this series with a mutation associated with the C15T codon in (4.5%) cases of the *inhA* gene. Furthermore, resistance to isoniazid carried by the *inhA* gene has been reported [2,30,31].

The present study reported 4 cases of multidrug resistance, of which the majority (14%) of mutations were observed at the S315T codon of the *katG* gene, followed by mutations at the H526D (9.1%) and H516D (6, 1%) of the *rpoB* and C15T genes (4.5%) of the *inhA* gene. Several previous research works have reported similar cases of multidrug resistance associated with mutations [14,32-34].

Two cases of resistance to rifampicin were observed in patients co-infected with tuberculosis and Hepatitis B in this series; the mutations occurred at the H526D codon of the *rpoB* gene. Cases of drug-resistant multidrug-resistant tuberculosis (MDR/RR-TB) in new cases (2.2%) by patients co-infected with HIV/TB have been reported elsewhere [35-37].

Conclusion

The results of this study show not only the resistance of *Mycobacterium tuberculosis* complex to rifampicin and isoniazid, but for the first time, cases of secondary resistance to (Levofloxacin, Amikacin, Kanamycin and Capreomycin) in Chad. Although resistant mutant strains of *Mycobacterium tuberculosis* are considered a major threat to tuberculosis control, these are epidemiological indicators of active disease transmission. In addition, the results highlight the interest of studies on drug-resistant tuberculosis. The data from this study should encourage national programs to activate the surveillance system for resistant *Mycobacterium tuberculosis* complex in endemic countries.

Conflict of Interests

The authors have no conflict of interest to declare.

Authors Contribution

All authors contributed significantly to the writing and editing of this manuscript. It has been seen and approved by all the authors. This manuscript has not been sent for publication elsewhere.

Acknowledgments

The authors would like to thank the Mycobacteria laboratory teams of the CHU-RN and the National Reference Laboratory of the National Tuberculosis Control Program (LNR-PNT) of Chad with its partners, namely the Global Fund, for providing the equipment, reagents and consumables necessary for carrying out this study.

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