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Research Article

Genotypic Profiles of *rpoB, katG* and *inhA* Gene Mutations Associated With *Mycobacterium tuberculosis* Resistance in Multidrug-Resistant Tuberculosis Patients in Niger

Profils Génotypiques des Mutations des Gènes rpoB, katG et inhA Associées à la Résistance de Mycobacterium tuberculosis chez des Patients Atteints de Tuberculose Multirésistante au Niger

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ABSTRACT

Introduction. In 2021, in Niger, among new cases, 9% of bacteriologically confirmed TB cases tested for rifampicin resistance; 81% of cases already treated and for all 79 cases of multidrug-resistant tuberculosis /rifampicin-resistant tuberculosis (MDR/RR-TB). The objective of this study is to determine the specific mutations in the *rpoB, katG* and *inhA* genes associated with the resistance of *Mycobacterium tuberculosis* to antituberculosis drugs in Niger. **Methods.** Sputum samples were collected from patients with rifampicin-resistant pulmonary tuberculosis and analyzed by the MTBDRplus 2.0 assay to determine the genetic profiles of mutations in the *rpoB*, *katG* and *inhA* genes associated with the multidrug resistance. **Results.** A total of 50 patients including 43 males (86%) were enrolled in the study. Mutations associated with multidrug resistance were detected in 6 patients (12%). They consist of S315T1 of the *katG* gene (30% of patients), C15T of the *inhA* gene (4% of patients) and H526D, S531L, H526D/H526Y of the *rpoB* gene (4% of patients each). Two areas of polymorphism (526-529 and 530-533) generated three types of mutations: MUT2A, MUT2B and MUT3 of which the combination of which was observed in 4% of patients. Three resistance genotypic profiles result from these mutations including RR-TB, HR-TB and MDR-TB. The TB-HR profile was predominant with 20% occurrence, but the TB-MR profile, observed in 12% of patients, remains worrying because it is more difficult to manage. **Conclusion.** Three genotypic profiles of resistance were observed, namely TB-RR, TB-Hr and TB-MR. The diagnostic strategy must now prepare the search of the TB-Hr genetic profile, which was moreover the most frequently detected in this study.

RESUME

Introduction. En 2021, au Niger, parmi les nouveaux cas, 9% des cas de tuberculose bactériologiquement confirmés ont été testés pour la résistance à la rifampicine ; 81% des cas déjà traités et pour l'ensemble des 79 cas de tuberculose multirésistante/tuberculose résistante à la rifampicine (MDR/RR-TB). L'objectif de cette étude est de déterminer les mutations spécifiques des gènes rpoB, katG et inhA associées à la résistance de Mycobacterium tuberculosis aux médicaments antituberculeux au Niger. **Méthodologie.** Des échantillons d'expectoration ont été prélevés chez des patients atteints de tuberculose pulmonaire résistante à la rifampicine et analysés par le test MTBDRplus 2.0 afin de déterminer les profils génétiques des mutations des gènes rpoB, katG et inhA associées à la multirésistance aux médicaments. **Résultats.** Au total, 50 patients, dont 43 hommes (86 %), ont été inclus dans l'étude. Des mutations associées à la multirésistance ont été détectées chez 6 patients (12%). Il s'agit de S315T1 du gène katG (30 % des patients), C15T du gène inhA (4 % des patients) et H526D, S531L, H526D/H526Y du gène rpoB (4 % des patients chacun). Deux zones de polymorphisme (526-529 et 530-533) ont généré trois types de mutations : MUT2A, MUT2B et MUT3 dont la combinaison a été observée chez 4% des patients. Trois profils génotypiques de résistance résultent de ces mutations : TB-RR, TB-HR et TB-MR. Le profil TB-HR était prédominant avec 20% d'occurrence, mais le profil TB-MR, observé chez 12% des patients, reste préoccupant car plus difficile à prendre en charge. **Conclusion.** Trois profils génotypiques de résistance ont été observés, à savoir TB-RR, TB-Hr et TB-MR. La stratégie diagnostique doit maintenant préparer la recherche du profil génétique TB-Hr, qui était d'ailleurs le plus fréquemment détecté dans cette étude.

HIGHLIGHTS

What is known of the subject

Globally, 3.3% of new TB cases and 17.7% of previously treated cases had RR-TB or MDR-TB. In Niger, estimates from the WHO show an incidence of RR/MR-TB of 2.6 (1.2-4.5)/100.000 inhabitants.

The aim of our study

Specific mutations in the *rpoB, katG* and *inhA* genes associated with the resistance of *Mycobacterium tuberculosis* to antituberculosis drugs in Niger.

Key Results

1. A total of 50 patients including 43 males (86%) were enrolled in the study. Mutations associated with multidrug resistance were detected in 6 patients (12%).
2. They consisted of S315T1 of the *katG* gene (30% of patients), C15T of the *inhA* gene (4% of patients) and H526D, S531L, H526D/H526Y of the *rpoB* gene (4% of patients each).
3. Two areas of polymorphism (526-529 and 530-533) generated three types of mutations: MUT2A, MUT2B and MUT3 of which the combination of which was observed in 4% of patients.
4. Three resistance genotypic profiles result from these mutations including RR-TB, HR-TB and MDR-TB. The TB-HR profile was predominant with 20% occurrence, but the TB-MR profile, observed in 12% of patients, remains worrying because it is more difficult to manage.

Implications for future practices and policies

The diagnostic strategy must now prepare the search of the TB-Hr genetic profile, which was moreover the most frequently detected in this study.

INTRODUCTION

Drug resistance is a barrier to TB mortality reduction [1] In 2020 at world scale 71% that is 2.1 million out of 3.0 million people with a bacteriologically confirmed diagnosis of pulmonary tuberculosis were tested for rifampicin resistance. Among these people, 132.222 cases of multidrug-resistant tuberculosis (MDR-TB)/rifampicin-resistant tuberculosis (RR-TB) were detected [2]. Globally, 3.3% of new TB cases and 17.7% of previously treated cases had RR-TB or MDR-TB [3]. In Niger, estimates from the WHO show an incidence of RR/MR-TB of 2.6 (1.2-4.5)/100.000 inhabitants. The estimated proportion of RR/MR-TB cases in 2019 is 2.5% (1.2-4.1) for new cases and 13% (10-16) for cases already treated [4]. The resistance to anti-tuberculosis agents is the result of simple mutagenic events that lead to amino acid substitutions in their target proteins [5]. MDR-TB can occur via two mechanisms: through the selection of resistant M. tuberculosis strains during a mismanaged anti-TB treatment or through direct transmission from one infectious patient to another [6]. An important factor driving the current MDR-TB epidemic is the direct transmission of MDR-TB strains, hence a high proportion of MDR-TB strains in previously untreated patients [7]. The WHO strategy to put an end to tuberculosis calls for early diagnosis of the disease, including the performance of universal drug susceptibility testing (DST). DST especially use genotypic (molecular) and phenotypic methods [8]. The molecular tool enabled to optimize the fight against tuberculosis by shortening treatment times, limiting the spread of tuberculosis, detecting rifampicin-resistant strains more quickly and facilitating the adaptation of anti-tuberculosis treatments [9]. The Xpert MTB/RIF system (Xpert; Cepheid, Sunnyvale, CA, USA) should be used as the initial diagnostic test for suspected MDR-TB or HIV-associated TB [10]. Indeed, more than 96% of rifampicin-resistant strains of M. tuberculosis harbor a mutation in the resistance-determining region (RDRR) of the rpoB gene [11]. Rifampicin resistance (RR) is a good indicator of multidrug resistance (MR) [12]. To detect MDR-TB, the *rpoB*, *katG* and *inhA* genes should be tested simultaneously. The WHO and the Global Laboratory Initiative (GLI) have approved and recommended the use of probe analysis tests (LPA, Hain Lifesciences, Nehren, Germany), the Genotype MTBDRplus test in this case [13; 14]. In 2021, in Niger, among new cases, 9% of bacteriologically confirmed TB cases tested for rifampicin resistance; 81% of cases already treated and for all 79 cases of MDR/RR-TB [4]. It is necessary to know the gene mutations and the genetic profiles carried by the tuberculous mycobacteria circulating in Niger. The objective of this study is to determine the specific mutations in the *rpoB*, *katG* and *inhA* genes associated with the resistance of *Mycobacterium tuberculosis* to antituberculosis drugs in Niger.

PATIENTS AND METHODS

Material

The study involves fifty 50) sputum samples collected from patients suspected of multidrug-resistant pulmonary tuberculosis presenting to GeneXpert units in Niger.
Method.

Study setting, type and population

The framework of this study is made up of GeneXpert units from six regions of Niger and the National Tuberculosis Reference Laboratory of Niger (NRL/TB) of Niamey. This is an analytical study during the year 2021 in the GeneXpert and NRL/TB units of the National Program for the Fight against Tuberculosis (PNLT).

Sampling

The technique of exhaustive recruitment of all patients with Rifampicin Resistant Tuberculosis (RR-TB) is used at the level of GeneXpert units in six regions of Niger in 2021. An exhaustive census of sputum samples from patients suspected of multidrug-resistant pulmonary tuberculosis is carried out at the level of units GeneXpert from six regions of Niger during the period from the beginning of June to the end of December 2021. After the Xpert/MTB/RIF test, samples were identified and retained from patients who were new cases resistant to rifampicin or with an indeterminate rifampicin result, patients in a retreatment situation regardless of the result of rifampicin.

Inclusion criteria

Included are patients with suspected multidrug-resistant tuberculosis (MR-TB) whose MTBDR*plus* Hain test is performed at the NRL/TB in Niamey.

Criteria for non-inclusion of cases

• Patients suspected of multidrug resistant tuberculosis who have already started anti-TB treatment for more than seven (7) days.

• Patients suspected of multidrug-resistant tuberculosis with extra-pulmonary tuberculosis.

• Patients suspected of multidrug-resistant tuberculosis who have not given their consent, patients whose LPA MTBDR*plus* molecular tests have not been done.

Studied variables

• Sociodemographic: age, sex, type of TB

• Risk factors: HIV test

• Molecular test results: MTBDR*plus* LPA test

Data collection tools and techniques

Data collection tools

The tools used to collect the data are the individual information sheets, the electronic Database (DB) of the GeneXpert units and the LPA test registers of the NRL/TB in Niamey.

Data collection technique

The data collection technique of [15] was respected. At Screening and Treatment Centers (CDT) and for each suspected patient whose smear is positive, two sputum specimens are collected before the start of treatment and sent to the GeneXpert unit. One of the two sputum is used for the Xpert/MTB/RIF test. For any patient whose test is MTB detected and rifampicin resistant or indeterminate or a case of retreatment regardless of the rifampicin result, the sputum sample is sent to the LNR-TB in Niamey for the LPA Hain MDR*plus* tests. This sample is packaged in a 50ml Falcon tube containing alcohol due to 2 volumes of pure 95° ethanol for 1 volume of sputum.

Methodology for researching mutations associated with resistance to antituberculosis drugs

The *rpoB* genes; *katG* and *inhA* were analyzed with the Genotype MTBDR*plus* VER 2.0 test. This test is based on DNA STRIP technology by reverse strip hybridization. It allows the identification of *M. tuberculosis* and resistance to rifampicin and/or isoniazid from clinical samples (sputum) or culture products. The identification of rifampicin resistance is done by the detection of the main mutations in the *rpoB* gene. The identification of isoniazid resistance is done by the analysis of *katG* and *inhA* genes. The Genotype MTBDR*plus* test (Hain life science, Nehren, Germany) was performed according to the manufacturer's instructions. The test is based on DNA strip technology and has three steps: DNA extraction, multiplex Polymerase Chain Reaction (PCR) and reverse hybridization. DNA was extracted using the Genolyse® kit (Hain Life Science GmbH, Nehren, Germany). For PCR, 10 ml of amplification mix A containing 10x buffer, nucleotides and DNA polymerase was mixed with 35 ml of amplification mix B containing MgCl2, primers and dye. The 5 ml of M. tuberculosis DNA was added to the mix, making the final volume of the PCR mix 50 ml. The amplification program is made up of 15 minutes of denaturation at 95°C, followed by 10 cycles of 30 seconds at 95°C and 120 seconds at 58°C, followed by 20 additional cycles of 25 seconds at 95°C, 40 seconds at 53 and 40 seconds at 70, with a final extension at 70°C for 8 minutes [16] (Wuyep *et al.*, 2019). For the hybridization, 20 ml of the amplification products were mixed with 20 ml of the denaturing reagent (provided with the kit) and the denaturation was carried out for 5 minutes in each of the plastic wells. Then 1ml of pre-warmed hybridization buffer was added to each well and one strip was placed in each well. Hybridization was carried out at 45°C for 30 minutes, followed by two washing steps. For colorimetric detection of hybrid amplicons, alkali phosphate-conjugated streptavidin was added, after which substrate buffer was added. After the final wash, the strips were air dried and fixed on paper supplied by the manufacturer.

DNA from standard strain H37RV and molecular grade water were used as positive and negative controls, respectively. The *rpoB, katG* and *inhA* gene loci each have a control band whose presence is mandatory for the interpretation of the results. The presence of mutations at the *rpoB* gene locus predicts resistance to RIF while mutations in the *katG* and *inhA* genes predict high-level and low-level resistance to INH, respectively. The absence of wild type and/or the presence of a mutant band signifies resistance to a particular drug [17; 16; 18] (Ahmed *et al.*, 2018; Wuyep *et al.*, 2019; Diandé *et al.*, 2019). The product leaflet was then consulted for the interpretation of the banding patterns and the establishment of the analysis report.

Statistical analysis

The data was processed with EpiInfo 7 software for the creation of the data collection mask and the performance of statistical tests.

Ethical considerations

This study received authorization from the National Ethics Committee for Health Research by Deliberation N°001/2019/CNERS of March 21, 2019.

RESULTS

The study included 50 sputum samples from patients with suspected multidrug-resistant pulmonary tuberculosis (MR-TB). Sputum from suspected MDR-TB patients was received and processed at GeneXpert units in six (6) regions of Niger from June to December 2021. Patient medical records were reviewed for age-related data, sex, HIV status and region of origin. The majority of patients come from two regions, namely Niamey 16/50 (32%) and Tillabéry 11/50 (22%) (Figure 1). Sociodemographic data showed that patients suspected of MDR-TB belong to the age group of 20 to 34 years (52%). The male gender predominates with 86% of patients suspected of MDR-TB. The majority of patients included in the present study have known HIV status and only 8% had TB/HIV co-infection.

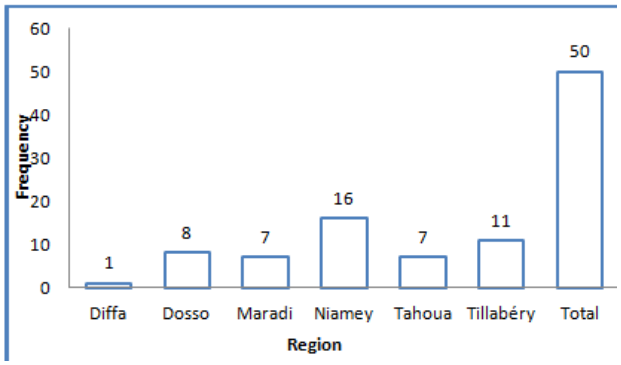

Figure 1. Distribution of patients by region

Gender and HIV status showed no significant association and the respective p-values are 0.146 and 0.706 with MR status (Table 1). Resistance to anti-tuberculosis drugs was detected in all age groups but without a significant association both for the two first-line molecules and for the MR profile (P-value = 0.320). Rifampicin and isoniazid have respective p-values of 0.160 and 0.288. All age groups are affected by rifampicin resistance (RR-TB) with the same frequency of 33% and this same frequency is found for the MR profile. But it is in relation to isoniazid that the 24 to 34 year old age group is the most affected with 63% (Table 2). The mutations were observed at the level of the three analyzed genes; 30% of patients had an S315T1 mutation carried by the *katG* gene at codon position 315.

Table 1. Socio-demographic characteristics of DR-TB and HIV patients and Relationship between gender, HIV status and MR profile

Variables	MR	Non MR	Total	P-value
Sex				
Female	2	5	7	
Male	4	39	43	**0.146 NS**
Total	6	44	50	
	20-34	26	52.0	[37.42%-66.34%]
Age (year)	35-49	17	34.0	[21.21% - 48.77%]
	≥50	7	14.0	[5,82% - 26,74%]
VIH Status	≥50	7	14,0	[5.82% - 26.74%]
Unknown	2	16	18	
Negatif	4	24	28	**0.706 NS**
Positif	0	4	4	
Total	6	44	50	

Table 2. Relationship between Age Rifampicin Isoniazid and MR.

Rifampicin				
Age range	R N(%)	S	Total	**P-value**
[20-34]	3 (33)	23	26	
[35-49]	3 (33)	14	17	**0,160 NS**
≥ 50	3 (33)	4	7	
Total	9 (100)	41	50	
Isoniazid				
Age range	R N(%)	S	Total	**P-value**
[20-34]	10 (63)	16	26	
[35-49]	3 (19)	14	17	**0.288 NS**
≥ 50	3 (19)	4	7	
Total	16 (100)	34	50	
MR				
Age range	R N(%)	S	Total	**P-value**
[20-34]	2 (33)	24	26	
[35-49]	2 (33)	15	17	**0.320 NS**
≥ 50	2 (33)	5	7	
Total	6 (100)	44	50	

For the *inhA* gene, only one type of mutation was observed at position -15 of its promoter. It is within the *rpoB* gene that two areas of polymorphism (526-529 and 530-533) have generated three types of mutations: MUT2A, MUT2B and MUT3. Moreover, 4% of patients are carriers of *M. tuberculosis* presenting a combination of three types of *rpoB* gene mutations: MUT2A, MUT2B, MUT3 (Table 3). Rifampicin resistance is associated with mutations in the RRDR (Rifampicin Resistance Determining Region) zone of the *rpoB* locus. Two types of mutational phenomena were observed: detected mutations 5/9 i.e 55% and deduced mutations 3/9 i.e 33%. The detected mutations are characterized by the absence of Wild Type (WT) and the presence of the codon-specific mutation. This is the case for mutations MUT2A, MUT2B for codons

526-529 and the MUT3 mutation for codons 530-533. The deduced mutations are, for their part, characterized by the double absence of WT and of the corresponding specific mutation (Table 4). In fact, this phenomenon is a deletion of the region of the genome with known resistance mutations. This is a rare mutation without mutation-specific capture probes.

Table 3. Mutations observed within the three *rpoB* genes; *katG* and *inhA*

Genes	Codons analyzed	Code Mutation	Mutations	N=50	%
rpoB	526 CAC → GAC	H526D	MUT2B	2	4.0
	531 TCG → TTG	S531L	MUT3	2	4.0
	526 CAC → GAC CAC → TAC 531 TCG → TTG	H526D/H526Y	MUT2A, MUT2B, MUT3	2	4.0
katG	315 AGC → ACC	S315T1	MUT1	15	30.0
inhA	-15 C → T	C15T	MUT1	2	4.0

Table 4. Mutations associated with RIF resistance within the *rpoB* gene N=9

rpoB	Frequency RIF-R N(%)	Profil TB
Absent WT8/MUT Absent	2 (22.0)	TB-RR
MUT2B	1 (11.0)	TB-RR
WT Present/MUT2B	1 (11.0)	TB-RR
MUT3	2 (22.0)	TB-RR
Absent WT7/MUT Absent	1 (11.0)	TB-RR
WT Present/MUT2A, MUT2B, MUT3	2 (22.0)	TB-RR

Table 5. Mutations and profile for resistant isoniazid (Hr = TB-RIF-S/INH-R), N=10

rpoB	***KatG* MUT**	***inhA* MUT**	RIF-S	INH-R	Profil TB	N(%)
MUT Absent	MUT1	-	S	R	TB-Hr	7 (70.0)
MUT Absent	-	Absent WT1/MUT Absent	S	R	TB-Hr	1 (10.0)
MUT Absent	MUT1	MUT1	S	R	TB-Hr	2 (20.0)

Table 6. Mutations associated with MR-type resistance

***rpoB* MUT**	***katG* MUT**	***inhA* MUT**	RIF	INH	Profil TB	N(%)
WT Present/MUT2B	WT Present/MUT1	Absent	R	R	TB-MR	1 (17.0)
MUT3	MUT1	Absent	R	R	TB-MR	2 (33.0)
WT absent/MUT absent	MUT1	Absent	R	R	TB-MR	1 (17.0)
MUT2A, MUT2B, MUT3	MUT1	Absent	R	R	TB-MR	2 (33.0)

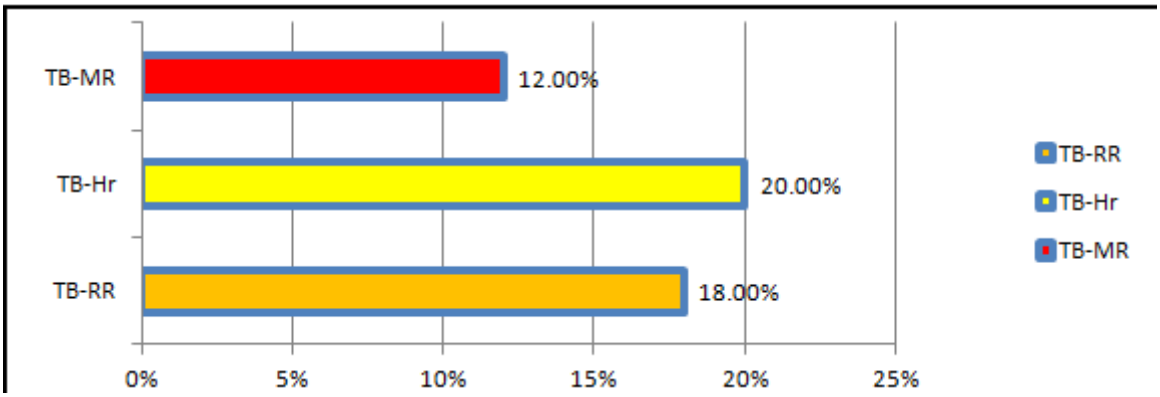

Figure 2. Resistance Genotypic Profiles Observed for Resistant Pulmonary Tuberculosis

The two types of such mutations observed in this study are the WT8 Absent/MUT Absent mutation and the WT7 Absent/MUT Absent mutation. Of all the observed deletion mutations, WT7 Absent/MUT Absent presented the lowest frequency which is 11%. Another type of mutation observed is the concomitant detection of the WT band and a MUT band. This type of situation characterizes heteroresistance, which is the coexistence of sensitive MTB strains and resistant MTB strains. 11% of patients have this type of mutation: WT Present/MUT2B. This result is considered resistance. Patients infected with MTB strains whose rpoB is mutated develop rifampicin-resistant tuberculosis (RR-TB). Resistance linked to the mutation can be interpreted in two ways: it is direct evidence in the case where the MUT band is present or indirect evidence in the case where the WT band is absent (Table 4). Mutations associated with isoniazid resistance occur in two different genes which are *katG* and *inhA*. These mutations can be observed simultaneously in both genes or only in one of the two genes. Mutations in *katG* induce high-level resistance while those in *inhA* are linked to low-level resistance to isoniazid. Some of the patients, i.e. 20%, in this study are carriers of MTB in which each of the two genes carries a MUT1 mutation corresponding to a high increase in the MIC detected for INH (Table 5). The MUT1 of the *katG* gene is a consequence of the codon 315 mutation and carries the code S315T1. As for the MUT1 of *inhA*, it intervenes at the level of the promoter at position -15 and carries the code C15T. It should be noted that 10% of patients are carriers of MTB presenting the AbsentWT1/MUT Absent type mutation within the *inhA* gene which produces the same effects as the presence of the MUT1 of *katG* (Table 5). Patients infected with MTB strains in which both genes (*katG* and *inhA*) or one of them is mutated, develop isoniazid-resistant tuberculosis (Hr-TB). Three genes are involved in multidrug resistance to anti-tuberculosis. First, the marker gene for this multiresistance, which is *rpoB*. A mutation observed within this gene implies the search for simultaneous or isolated mutations within the *katG* and *inhA* genes. Six of the fifty patients (12%) in this study are carriers of MTB strains with mutations associated with multidrug resistance (Table 6). 33% of patients are infected with MTB strains having a triple mutation of the *rpoB* gene and 17% are carriers of MTB having undergone a deletion of the WT8 band corresponding to codons 530-533. A particular case is found in 17% of patients where the mutation is of the *rpoB* WT Present/MUT2B and *katG* WT Present/MUT1 type. In this case, it is a mixed or double population due to the presence of sensitive strains and resistant strains vis-à-vis the two anti-tuberculosis drugs, rifampicin and isoniazid. All these patients are carriers of MTB with a mutation associated with a high level of resistance to isoniazid by raising the MIC, that is to say a mutation at the level of the *katG* locus. (Table 6). Tuberculosis resistance is characterized by different types of profiles, three of which were observed in this study. The TB-Hr profile, with 20%, presents the highest frequency but the profile of greatest concern and the most difficult to manage is the TB-MR profile. This profile was observed in this study with a frequency of 12% (Figure 2).

DISCUSSION

With Fifty (50) sputum samples from patients suspected of resistant pulmonary tuberculosis were collected at six (6) GeneXpert Units in Niger from June to December 2021.

Origin of patients

The Niamey region with 16/50 (32%) provided the largest number of suspected MDR-TB patients. This situation could be explained by the fact that Niamey is home to national hospital structures involved in the fight against tuberculosis in general and resistant tuberculosis in particular. It is within the National Anti-Tuberculosis Center of Niamey that the main TB-MR Unit is located. Another asset for the Niamey region is the presence of the LNR-TB which is equipped with all the classic and modern infrastructures for the biological diagnosis of MDR-TB.

Socio-demographics characteristics

The age group of 20-34 years with 52.0% (95% CI: 37.42% - 66.34%) is the most affected by multidrug-resistant tuberculosis. The predominance of this group is mentioned in the Niger profile report by WHO in 2021 where out of the cumulative 11,552 cases of declared tuberculosis, the age group from 20 to 34 years represented 6,300 cases i.e 54.53% [19]. This result contrasts with the one of Kabir in 2021 in Pakistan who found that MR patients belong to the age group of 21 to 40 years (78.2%) [20]. According to the WHO, the majority of people infected with MR-TB are between 25 and 44 years old. The predominance of young subjects can be explained by non-compliance with the relatively long treatment protocol (9 to 18 months) in this age group considering that they are very preoccupied, among other things, with studies, work [20]. Sociodemographic data showed no significant association between multidrug-resistant tuberculosis (MDR-TB) and anti-tuberculosis drugs according to age or sex. Regardless of the type of resistance, male subjects predominate. This predominance is clearer for resistance to INH, i.e. 14/16 (87.5%). INH resistance is more common in men with a sex ratio of 7. This kind of situation is reported by studies that show ratios ranging from 1.5 to 8 in favor of men [21]. A significant association was found between RR-TB and gender, p-value 0.004 but not significant for MDR-TB where the p-value is 0.146. This situation contrasts with that described in the study by [20]. Gender does not influence the acquisition of resistance to anti-tuberculosis drugs. The male predominance observed in tuberculosis patients with resistance can be explained by the predominance of tuberculosis in male individuals. Gender is not a factor that increases the probability of having resistance. The male predominance can be explained by the hypothesis that women are more compliant with treatment and therefore less likely to receive inadequate treatment [20]. HIV infection may contribute to the risk of MDR-TB through a range of potential mechanisms [22]. But in this study no

significant association was found between RD patients and HIV, p-value 0.706.

Observed Mutations

Within the RDRR region of the *rpoB* locus, the most frequent mutation detected is MUT3B, i.e. 22%, which contrasts with the results of LUO in China where the most frequent mutation was of the *rpoB* 531 type. Three types of resistant tuberculosis were detected identified during this study. But the TB-Hr profile presents the highest frequency, i.e. 20%, compared to the other two which are TB-RR 18% and TB-MR 12%. This result suggests that testing for rifampicin resistance is not necessarily the best approach for the diagnosis of probable MDR-TB [23]. Rifampicin-resistant tuberculosis was detected in 18% of patients. This rate is significantly higher than the 5.9% rate found in Maiduguri in Borno State in Nigeria [24]. INH resistance [25] (Luo et al., 2019) usually accompanies RIF resistance. Rifampicin resistance is an extremely sensitive marker of multidrug-resistant tuberculosis (MDR-TB) [26; 27; 28]. The rate of MDR-TB found in this study is 12%. This rate is very high compared to that published in the Niger profile by WHO in 2021, which is 3% (1-4%) for MDR/RR tuberculosis [29]. This difference could be explained by the fact that this rate is obtained from a target population suspected of resistant tuberculosis. Among the three anti-biotypical profiles of tuberculosis identified in this study, MDR-TB comes with the lowest frequency that is 12%. This profile of tuberculosis is the one that poses the most difficulties in its therapeutic management. Indeed, the two first-line anti-tuberculosis drugs, rifampicin and isoniazid, are no longer effective. Second-line anti-tuberculosis drugs should be used. The seriousness of the therapeutic management of MDR-TB is due to the fact that the two antituberculosis drugs RIF and INH, which have become ineffective, have been the subject of several mutational phenomena, each within it. These two molecules do not have the same resistance mechanisms, but also their mechanisms of action are not identical. Indeed, rifampicin and isoniazid do not act in the same way on *M. tuberculosis*. INH has potent bactericidal activity against strains of *M. tuberculosis*. It is a prodrug activated by the KatG enzyme of *M. tuberculosis*, which is a catalase-peroxidase. INH inhibits mycobacterial cell wall synthesis, leading to cell death. About 80% of INH-resistant strains carry point mutations or partial or complete deletions of the *katG* gene. Resistance to RIF is conferred by mutations in the *rpoB* gene. Some strains may be resistant to RIF but sensitive to INH, leading to their misclassification as MR strains if testing of MR strains is based exclusively on RIF resistance [23]. Of the six cases, five are new cases and this situation is contrary to that found by Sylverken in Ghana. Indeed, retreatment is known to be the strongest determinant of MDR-TB cases [28]. The algorithm for diagnosing resistant tuberculosis in Niger (TB-RR/TB-MR) has, first of all, to look for resistance to rifampicin and, in the event of positivity, to check for simultaneous resistance to rifampicin and to isoniazid. However, the results of this study reveal a significant proportion of TB-Hr, i.e. monoresistance to INH. These cases are beyond the provisions of the algorithm considering that the search for resistance to INH is conditioned by the positive detection of resistance to rifampicin. It is now becoming useful and necessary to include the search for the INH profile in addition to the search for possible antibiotic profiles, signs of resistant tuberculosis.

CONCLUSION

The use of molecular tests such as the MTBDR*plus* makes it possible to better control the problem of resistant tuberculosis in Niger. This study enabled to detect point mutations in the three essential genes involved in resistance to antituberculosis drugs. Some mutations were nucleotide substitutions within polymorphic codons while other mutations were deletions in regions of these genes. The observed mutations enable to detect resistance or to deduce it. One of the important advantages of this molecular test is its ability to highlight patients infected with MTBs comprising two subpopulations, one of which is sensitive while the other is resistant. Three genotypic profiles of resistance were observed, namely TB-RR, TB-Hr and TB-MR. The diagnostic strategy must now envisage for the search for the TB-Hr genetic profile, which was moreover the most frequently detected in this study.

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Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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