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Occurrence of mutations associated with rifampicin and isoniazid resistant in *Mycobacterium tuberculosis* isolates from patients in Burkina Faso

Abstract

Genetic mutations are responsible for the high rate of resistance observed in the treatment of tuberculosis. This study aimed at determining the occurrence of mutations associated with rifampicin (RIF) and isoniazid (INH) resistance of *Mycobacterium tuberculosis* complex (MTBC) isolates. MTBC strains isolated by culture from 110 TB patients diagnosed with resistant to rifampicin (RR-TB) by Xpert MTB/RIF were studied. The isolates were obtained from the National Tuberculosis Reference Laboratory in Ouagadougou. They were identified culturally using Antigenic method (SD Bioline TB Ag MPT64). Polymerase Chain Reaction, PCR (*DRplus*) was used to detect the occurrence of mutations in the genes associated with resistance *katG and inhA* promoter for INH, and *rpoB* for RIF. Out of 103 isolates with RIF resistant, mutations were detected in 87(84.5%) of gene *rpoB* while no mutation was found in 16(15.5%) of the gene of the isolates even though the wild probes had disappeared. Single mutations were found in the codons D516V (41.7%) and H526Y (17.5%) while combined mutations (single and double) were mostly detected in the codons D516 (51.5%), H526Y (20.4%), S531L (11.7%) and H526D (10.7%) respectively. Single mutations responsible for high-level isoniazid resistance, *katG* were observed in the codon S315T1 while the combined *inhA* and *katG* were detected in the codon C8T and S315T, 16 (14.5%) respectively. The highest mutation occurrence was observed with *rpoB*516, *rpoB*526 for RIF and *katG*315 for INH associated with resistance of MTBC isolates. There is a need to improve molecular assay kit diagnosis to curb the geographic specificity of the target genes needed to detect more possible mutations.

Keywords: *mycobacterium tuberculosis* strains, genes, mutations, resistance

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Introduction

The emergence of multidrug resistant (MDR) strains of MTBC has become one of the most critical issues for tuberculosis (TB) control programmes worldwide. It is a public health concern threatening global TB control programs. Its diagnosis has evolved in recent years following the development of new molecular techniques based on detection of mutations in MTBC genes by Polymerase Chain Reaction (PCR).[1–3]

The Genotype *MTBDRplus* is a commercially available molecular gene Line Probe Assay developed by Hain Life Science, (Nehren, Germany). It is performed on MTBC isolates or directly from clinical specimens. It able to identify the MTBC and detect the genetic mutations in the *rpoB* gene related to rifampicin resistance, the *katG*, *inhA* regulatory region and *inhA* genes related to isoniazid resistance. Its targets points are the 81-bp "hot spot" region of the *rpoB* gene of RIF, codon 315 of *katG* and *inhA* promoter regions of INH.[4–7]

The genetic basis of multidrug resistant MTB isolates has been widely studied worldwide and commonly believed to be caused by point mutations in important genes like *rpoB* and *katG*. Multiple studies carried out at different time periods in the same country/geographical setting have yielded variable incidence of specific *rpoB* mutation.[8, 9]

In Burkina Faso, the fight against MDR-TB/rifampicin-resistant tuberculosis (RR-TB) has become a National concern. For this purpose, the technical platform of the National Reference Laboratory (NRL) for *Mycobacteria* in Ouagadougou was strengthened with Molecular tests such as Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) and Line Probe Assay (Hain Life Science GmbH, Nehren, Germany). However, other fourteen peripheral Laboratories in the Country have also been equipped with GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA). So, the guidelines of the National Tuberculosis Control Program recommend the use of *DRplus* and *DRsl* for all TB-patients confirmed RR-TB by Xpert test (Cepheid, Sunnyvale, CA, USA).

This study was to determine the occurrence of specific *rpoB, katG and inhA* gene promoters' mutations in rifampicin and isoniazid resistant *M. tuberculosis* isolates from TB-patients in Burkina Faso.

Materials and methods

Study area and laboratory analysis

We studied rifampicin resistant *M tuberculosis* strains isolated from 110 TB patients diagnosed with resistant to rifampicin (RR-TB) by Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) at the NRL in Ouagadougou. The patients were undergoing treatment at Centres for Diagnostic Tuberculosis (CDT) between 2014 and 2016 in the 13 Health regions of Burkina Faso. During this period, suspected MDR-TB patient's sputa were collected from the various CDTs in the Health regions of the Country and transported to the NRL in Ouagadougou where they were identified culturally using Antigenic method (SD Bioline TB Ag MPT64); thereafter, PCR (*DRplus)* confirmation. The patients' medical records were review to obtain relevant data on the age, sex, category of patients, HIV status, and region of origin.

Molecular analysis by genotype MTBDR plus 2.0

One hundred and ten (110) *Mycobacteria tuberculosis* isolates were

selected for the Molecular analysis at the NRL. DNA was extracted using Genolyse® kit (Hain Life Science GmbH, Nehren, Germany). The extracted DNA was processed by the LPA using *DRplus* (Hain Life Science GmbH, Nehren, Germany) to detect MTBC and RIF and/or INH resistance according to the manufacturer's instructions.[7] PCR amplification was carried out using biotin-labeled primers. The colorimetric detection of the strips was carried out using enzymes in which DNA products were bound to the strip. Positive internal quality control and negative control were used during the tests. The *rpoB*, *katG*, and *inhA* gene loci each have a control band, the presence of which is mandatory for results interpretation. The presence of *rpoB* gene locus predicts RIF's resistance while *katG* predicts high level and *inhA* low-level INH resistance. Absence of wild type and/or presence of mutant band mean resistance to a particular drug. The product insert was further referred for interpretation of banding patterns and trouble shooting.

Limitation of the study

We have not reported the occurrence of *katG* and *rpoB* mutations in INH and RMP resistant *M. tuberculosis* isolates from patients of different ethnic background from Burkina Faso. Secondly, among the isolates studied, we do not know which Beijing genotype strains are or not. These two limits did not allow us to understand the higher frequencies of certain mutations compared to the studies that studied them. Thirdly, only *DRplus* was used to study the occurrence genes mutations.

Statistical analysis

The results obtained were entered into Statistical Program SPSS version 20.0 and the frequency of patient characteristics and mutations in the *rpoB*, *katG* and *inhA* genes calculated

Results

Patients' characteristics

One hundred and ten (110) TB patients with resistant to rifampicin by Xpert test (27 females and 83 males) were included. The average age for female patients was 39.9 years (14–70 years), while the male was 36.5 years (16-86 years). Two (2) females and 6 males were tested HIV positive and 26 females and 79 males had a history of TB treatment or were undergoing TB treatment (Table 1). *DRplus* revealed 76 patients who were resistant to rifampicin, 83 to isoniazid. Among the females, 27 patients were resistant each to rifampicin and isoniazid while 24 were resistant to both isoniazid and rifampicin respectively. The results of Xpert test and *DRplus* were discordant for 7 patients. This is as presented in Table 1.

Occurrence of mutations in isoniazid and rifampicin resistance associated targets

In Table 2, out of the 103 isolates with RIF resistant, single mutations were observed in the codons D516V (41.7%), followed by H526Y (17.5%) and then S531L (5.8%). Double mutations in *rpoB* gene were observed in 6 of the codons D516V+S531L (5.8%), 3 of D516V+H526Y (2.9%) and 1 of D516V+H526D respectively. The occurrence of single and combine mutations in *rpoB* gene where found to be 51.5% in the codons D516V, 20.4% in H526Y, 11.7% in S531L and 10.7% in H526D respectively while no mutation was found in 16 (15.5%) other cases, even though the wild probes had disappeared.

Table 1 Patients characteristics

Characteristics	Sex	
	Male (%)	**Female (%)**
Number of patients	83 (75.5)	27 (24.5)
Age	36.5 (16 - 86)	39.9 (14 – 70)
HIV positive	6 (75.0)	2 (25.0)
HIV negative	75 (75.8)	24 (24.2)
With TB treatment history	79 (80.0)	26 (20.0)
New patients	4 (73.8)	1 (26,2)
RIF- resistance	76 (75.5)	27 (24.5)
INH-resistance	83 (75.5)	27 (24.5)
Both RIF-INH-resistance	72 (75.0)	24 (25.0)

RIF: rifampicin; INH: isoniazid

The mutations responsible for high-level isoniazid resistance in *katG* gene were observed in the codons S315T1 (77.3%), S315T2 (2.7%), unknown (3.6%) while combined high-level isoniazid resistance mutations in *katG* and *inhA* gene where observed in the codons C8T+S315T (14.5%) respectively. The mutations responsible for a low level of resistance to isoniazid in *inhA* were observed in the codons C8T+C15T (0.9%) with unknown mutation (0.9%).

Occurrence of codon mutations in rifampicin and isoniazid resistant isolates from different countries/ geographical settings

The comparison of the occurrence of codon mutations in rifampicin and isoniazid resistant MTBC isolates from different geographic regions is presented in Table 3 & 4 respectively. The frequency of

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mutations in the *rpoB* gene was found in the codons 516, 526 and 531 varied in different geographic regions (Table 3). It is the same for the occurrence of mutations in the *katG* gene and *inhA* gene promoter (Table 4).

Table 2 Occurrence of mutations in *Mycobacterium tuberculosis* genes (*rpoB, katG* and *inhA*) associated to rifampicin and isoniazid resistance

Resistance to drugs					**Resistance no drug**	**%**
RIF-résistance (rpoB gene)					N = 103	%
WT probes		Mutant probes				
		S531L			6	5.8
ΔWT3/4		D516V			43	41.7
ΔWT7		H526Y			18	17.5
		D516V + H526Y			3	2.9
		D516V + H526D			1	1
ΔWT3-8		D516V + S531L			6	5.8
ΔWT8		H526D			3	2.9
ΔWT7/8		H526D			7	6.8
ΔWT8		unknown			6	5.8
ΔWT7		unknown			5	4.9
ΔWT2/7		unknown			1	1
ΔWT2/3		unknown			1	1
ΔWT3-4-8		unknown			1	1
ΔWT3-8		unknown			2	1.9
					N=110	%
INH Resistance						
katG		**inhA**				
WT probes	Mutant probes	WT1 probe	WT2 probe	Mutant probes		
ΔWT	S315T1				85	77.3
ΔWT	S315T1		ΔWT2	T8C	16	14.5
ΔWT	unknown				4	3.6
ΔWT	S315T2				3	2.7
			ΔWT2	T8C	1	0.9
		ΔWT1/2	C15T+	T8C	1	0.9

Table 3 Occurrence of codon mutations in rifampicin-resistant *M. tuberculosis* isolates from different countries/geographical settings

Countries	**Year**	**n**	**531**	**516**	**526**	**Ref**
Burkina Faso	2019	103	0 (0.0)	43 (41.7)	28 (27.2)	Our study
China	2018	79	46 (58.2)	8 (10.1)	10 (12.7)	22
Kyrgyzstan	2018*	185	120 (64.8)	15 (8.1)	32 (17.3)	23
Punjab (India)	2017	137	80 (58.4)	8 (5.8)	12 (8.7)	24
Ethiopia	2017*	49	40 (81.6)	1 (2.04)	4 (8.16)	25
Ghana	2017*	13	2 (15.4)	6 (46.2)	3 (23.1)	11
Brasil	2016**	43	27 (62.8)	-	3 (7.0)	15
South West Ethiopia	2016**	34	28 (82.4)	-	1 (2.9)	16
Ivory Coast	2016	60	11 (18.3)	23 (38.3)	15 (25.0)	12
Nigeria	2015**	10	5 (50.0)	2 (20.0)	3 (30.0)	6

Table Continued

Countries	Year	n	531	516	526	Ref
Ivory Coast	2014	95	16 (16.8)	21 (22.1)	27 (21.1)	13
Georgia	2013	634	426 (67.2)	33 (5.2)	20 (3.2)	18
Taiwan	2013*	22	68.2	4.5	4.5	26
North India	2012*	30	16 (53.3)	3 (10.0)	5 (16.7)	2
Ethiopia	2012*	15	11 (73.3)	-	1 (6.7)	21
Shanghai (China)	2010*	242	143 (59.1)	12 (5.0)	13 (5.4)	27
Burkina Faso	2009	32	3 (9.4)	14 (43.7)	10 (31.2)	10
Samara (Russia)	2009	107	93 (86.9)	1 (0.9)	2(1.8)	28
Taipei, Taiwan	2009	2031	146 (63.2)	6 (2.6)	10 (13.5)	29

*: only H526Y; **: only H526D

Table 4 Occurrence of codon mutations in isoniazid-resistant *Mycobacterium tuberculosis* isolates from different countries/geographical settings

Country	Year	Number	katG		inhA		Reference
			S315T1	S315T2	C8T	C15T	
Our study		110	85 (77.3)	3 (2.7)	1 (0.9)	0	
China	2018	65	44 (67.7)	1 (1.5)	-	13 (20.0)	22
Kyrgyzstan	2018	104	91.2		-	8 (7.0)	23
Punjab (India)	2017	134	110 (82.1)	1 (0.7)	3 (2.2)	17 (12.7)	24
Ethiopia	2017	52	52 (100.0)	-	-	-	25
Ghana	2017	29	24 (82.8)	-	6 (20.7)	5 (17.2)	11
South West Ethiopia	2016	41	36 (87.8)	0 (0.0)	-	4 (9.8)	16
Brasil	2016	43	18 (41.9)		-	11 (25.6)	15
Ivory Coast	2016	59	59 (100.0)		16 (27.1)	-	12
Nigeria	2015	7	3 (42.9)	-	-	-	6
Ivory Coast	2014	120	76 (63.3)	-	24 (20.0)	1 (0.8)	13
Georgia	2013	634	535 (84.3)		8 (1.3)	143 (22.6)	18
Ethiopia	2012	35	33 (94.3)	-	-	2 (5.7)	21
Burkina Faso	2009	36	36 (100)		7 (19.4)	3 (8.3)	10
Russia	2009	117	68 (58.1)	1 (0.9)	-	3 (2.6)	28
Taipei, Taiwan	2009	198	96 (48.5)	1 (0.5)	8 (3.5)	60 (30.3)	29

Discussion

This study evaluated the occurrence of mutations in the *rpoB*, *katG*, and *inhA* gene promoters responsible for the resistance of *M. tuberculosis* complex to rifampicin and isoniazid in patients detected RR-TB by Xpert MTB/RIF. It revealed mutations in the *rpoB* gene most commonly at codons D516V, H526Y and S531L. The codon 516 (51.5%) had the highest mutations in the *rpoB* genes. Such a high occurrence agrees with the reports of previous studies in Burkina Faso and other parts of the world; such as[10] who reported 43.7% in Burkina Faso[11] who reported 46.2% in Ghana.[12] It is possible that the epidemiology of TB is identical for these Countries. However, the finding was at variance with[5,13,14] who reported a low occurrence of 2.1%, 20.0% and 21.1% respectively. Regional variation in the epidemiology of TB could be accounted for these discrepancies.

On the other hand, studies carried out in South West Ethiopia and in Basil have not found a mutation at codon 516.[15,16] The occurrence of 531 mutations (11.7%) in this study was comparable to 9.4% found in the previous study.[10] Our finding and those of Burkina Faso neighboring countries [11,12–14] contrast enormously with the results of numerous studies[15–29] which reported higher rates of mutations at 531. High rates of 531 mutations were particularly found in Kyrgyzstan, Ethiopia, Brasil, South West Ethiopia, Georgia, Taiwan and Samara in Russia.[21,23–28] Some authors attributed the variations in the frequency of *rpoB*-specific mutations to the geographical differences in RMP-resistant *M. tuberculosis* strains circulating in different settings and their clone propagation.[28,30] So, for Taiwan, codon 531 accounted for 68.2% of mutations could be due to the spread of a prevalent genetic clone.[26] The high occurrence of *rpoB*531 mutation in MDR-TB strains from Samara region in Russian Federation was attributed to the high frequency of Beijing genotype strains,[28] but that's not a certainty. For instance, the frequency of *rpoB*531 mutations is much lower in RMP-resistant strains in China where the frequency of the Beijing genotype

strains is high.[31] The occurrence of mutation at 526 reported in the current study is higher than rate found in Burkina Faso neighbouring Countries,[11,12–14] lower than the value reported in Nigeria[6] and similar to findings reported from India and Kyrgyzstan.[2,21] All this indicates that mutations at codons 531, 526 and 516 are common, with a varied occurrence across the geographical areas.

In this study, occurrence of gene mutation attributed to low level drug resistance mainly caused by the mutations in the promoter region of *inhA* gene was lower than the mutation frequent C15T observed in Ivory Coast.[13] Occurrence of *katG*315 mutations was lower than those obtained in Ghana and in Southwest Ethiopia,[11,25] similar to an Ivorian study[12], and higher than a report from Nigerian.[6] The high occurrence (90-95%) of *katG*315 mutations among *M. tuberculosis* strains in Russia was attributed to the high frequency of Beijing genotype strains.[32,33] However, the frequency of *katG*315 mutations is much lower in INH resistant strains in Taiwan, where the frequency of the Beijing genotype strains is high.[34,35] Some authors reported variations of the occurrence of *kat*G315 mutations in isoniazid-resistant isolates among patients of different ethnic groups at the same geographic locations.[8] It remains to be elucidated whether these differences of variations are due to differences in the genetic background of *M. tuberculosis* isolates or to differences in ethnic origin of the infected TB patients or both.

Our study showed higher level of rifampicin-resistant isolates with no mutation. It corroborated with similar reports from elsewhere.[17,36,37] It is possible that less common mutations in *rpoB* gene cannot be detected by *DRplus*.[21] In addition, the possibilities of absence of mutation in RRDR of rpoB gene in MDR-TB isolates may be due to existence of other rare *rpoB* mutations outside RRDR or different mechanism of rifampicin resistance.[38] For strains classified as isoniazid-resistant with no mutation, it is known that about 10% to 25% of isoniazid-resistance strains are thought to have mutations outside *KatG* and *inhA loci*. Efflux system may play such a role.[39,40] To cater the geographic specificity of the target genes and be able to detect more possible mutations in different geographical areas, it is necessary to improve molecular assay kit diagnosis.[41,42]

Conclusions

In Burkina Faso, the general pattern of *rpoB*, *katG* and *inhA* mutations observed is similar to that reported globally in most clinical *M. tuberculosis* isolates. The highest mutation occurrence was observed respectively at 516 and 526 of *rpoB*, and *katG* (S315T1) genes. However, the *DRplus*, did not identify certain mutations while the wild probes had disappeared. So, the mutations identified are useful as molecular markers for the detection of multidrug resistant isolates but are not yet sufficient to fully predict a multidrug resistance of *M. tuberculosis*. Hence the need to improve molecular assay kit diagnosis to cater for the genetic variations associated with the geographic specificity of the target genes and be able to detect most frequent mutations in different geographical areas. Likewise, using phenotypic drug susceptibility testing and sequencing will allow identifying missing mutations not detected by the *DRplus*.

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None

Conflicts of interest

The authors declare no conflicts of interest.

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