Letter to the Editor

Frequency of mutations in the *rpoB* gene of multidrug-resistant Mycobacterium tuberculosis clinical isolates from Sudan

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Multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* represent an obstacle in tuberculosis control and stress the need for rapid and reliable diagnostic tools for drug susceptibility testing in clinical isolates [1]. Mutations in the 81-bp region of the *rpoB* gene are responsible for resistance to rifampicin, an essential drug for the therapy of tuberculosis (TB) [2]. Mycobacteria laboratory facilities in Sudan exist only in Khartoum state whereas TB patients from other states are referred to Khartoum state for diagnosis and treatment. The MDR-TB prevalence in Sudan was found in 5% of new cases and 24% of previously treated patients [3]. Recent development of the Xpert MTB/RIF test [4] and of the line probe assay [5] which span 81-bp fragment of the RNA polymerase beta subunit (*rpoB*) gene have allowed for the rapid detection of resistance to rifampicin (RIF). Knowledge on the geographical distribution of the *rpoB* resistant alleles is essential for the development of diagnostic tools. Although sequence analysis of the *rpoB* gene has been shown to be effective for detecting RIF-resistance alleles in over 90% of RIF-resistant isolates from different geographical regions [6,7], there is only limited information from Sudan [8]. In order to find Sudan-specific mutations, that could potentially be used for the optimization of the current detection tools of RIF resistance alleles, we determined in this study the mutation profile occurring within an 81-bp fragment of the *rpoB* gene, in a collection of MDR clinical isolates of *Mycobacterium tuberculosis* isolated from Khartoum, Sudan.

Forty-nine clinical isolates consisting of RIF-resistant *M. tuberculosis* were isolated from sputum of TB patients during the period between 2007 and 2009, into the Khartoum state, Sudan. The drug susceptibility testing was performed using standard proportional method and the drugs tested were rifampicin, isoniazid (INH), ethambutol (EMB), and streptomycin (SM) [9].

Genomic DNA was extracted using the boiling method. An 81-bp region of the *rpoB* gene was amplified by polymerase chain reaction (PCR) [10] and DNA sequencing was performed by Macrogen Inc. (Seoul, South Korea) on a ABI3730XL DNA sequencer (Applied Biosystems, Foster City, USA). Water was used as a negative control to replace DNA, to rule out carry-over contamination of the amplicon throughout the steps of the protocol.

We report here the determination of the *rpoB* gene mutation profile in MDR *M. tuberculosis* clinical isolates from the Khartoum state and the comparison with mutation profiles from different countries. The resistance profile and mutations of *rpoB* are listed in Table 1. The mutations observed in this study were at codon 531 (64.1%), 526 (17.9%), 516 (7.7%), 511 (2.6%) and two isolates (5.1%) had insertion between codon 514-515. All genetic alterations were caused by single base substitutions, and the most frequent mutation was observed at codon 531 (Ser ⇒ Leu 61.5%). Missense mutations with different frequencies were observed at codon 526 (His ⇒ tyr 12.8%), (His ⇒ leu 5.1%) and (His ⇒ cys 2.5%). Other mutations were at codon 531 (ser ⇒ Trp 2.5%), 516 (asp ⇒ val 7.6%) and two isolates had insertion of Phe. Allele10 contained novel mutation at codon 511 (CTG leu to CCC pro) that has never been previously reported. The sensitivity of *rpoB* mutations within the 81-bp region for predicting the RIF-resistance of *M. tuberculosis* is 80%. The new allele sequence has been deposited in GenBank under the accession number KF877732.

A comparison of mutation frequency at codon 531, 526, 516 and 511 was similar to mutation frequency reported from Taiwan, India, Italy and China [11,12,13,14]. Mutations within the 81-bp hot-spot region (codons 507 to 533) account for more than 90% of RIF-resistant *M. tuberculosis* strains [6,7], but still there was a proportion of strains phenotypically RIF-resistant but lacking mutation within the hot-spot region. Yue *et al*., 2003 [14] have reported that 10% of phenotypically RIF-resistant *M. tuberculosis* isolates did not show any mutations at the 81-bp region. According to Adikaram *et al*., 2012 [15], 41.9% of RIF-resistant *M. tuberculosis* isolates contained mutation outside the hot-spot region. In the current study, 20% of our isolates contained no mutations within the 81-bp region. This disagreement between phenotypic resistant and genotypic sensitive isolates can be explained by occurrence of mutation elsewhere outside 81-bp region as shown by Adikaram *et al*., 2012 [15], or may be due to other mechanism. The 80% sensitivity obtained herein for *rpoB* mutations within the 81-bp region for predicting the RIF-resistance of *M. tuberculosis* was lower than the sensitivity reported from other countries which was > 90 %.

We conclude that the discovery of one new mutation and isolates lacking mutations mentioned herein emphasizes the need for mutations surveillance prior to the implementation of commercial molecular tools [4], and optimization of these techniques to cover different alleles of *rpoB* gene in Sudan. In addition, the detection of RIF-resistance by molecular technique [4] must always be confirmed by phenotypic methods in case of absence of mutations. Study limitations include the relatively short region of *rpoB* sequenced and the small sample size. Despite these limitations, this work characterizes the *rpoB* mutations in MDR TB in Sudan. In addition, our finding is valuable for the design of screening diagnostic tests for rapid detection of mutations associated with rifampicin resistance in Sudanese clinical isolates.

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Table 1. Frequency of *rpoB* mutation and antibiotics profile in MDR *M. tuberculosis* isolates

Alleles	Codon	Amino acid substitution	Mutation	No of isolates	RIF	INH	EMB	SM
Allele 1	531	ser⇒Leu	tcg⇒ttg	23	R	R	R	R
Allele 2	531	ser⇒Leu	tcg⇒ttg	1	R	R	S	R
Allele 3	531	ser⇒Trp	tcg⇒tgg	1	R	R	R	R
Allele 4	526	His⇒tyr	cac⇒tac	5	R	R	S	S
Allele 5	526	His⇒leu	cac⇒ctc	2	R	R	R	R
Allele 6	526	His⇒cys	cac⇒tgc	1	R	R	R	R
Allele 8	516	asp⇒val	gac⇒gtc	3	R	R	R	R
Allele 9	514-515	phe	insertion ttc	2	R	R	R	R
Allele 10	511	leu⇒pro	ctg⇒ccg	1	R	R	R	R
Allele 11	-	-	No mutation within 81-bp fragment	4	R	R	R	R
Allele 12	-	-	No mutation within 81-bp fragment	2	R	R	S	S
Allele 13	-	-	No mutation within 81-bp fragment	3	R	R	S	R
Allele 14	-	-	No mutation within 81-bp fragment	1	R	R	R	S

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