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Mutations inside rifampicin-resistance determining region of *rpoB* gene associated with rifampicin-resistance in *Mycobacterium tuberculosis*

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ABSTRACT

Background: Rifampicin (RIF) plays a pivotal role in the treatment of tuberculosis due to its bactericidal effects. Because the action of RIF is on *rpoB* gene encoding RNA polymerase β subunit, 95% of RIF resistant mutations are present in *rpoB* gene. The majority of the mutations in *rpoB* gene are found within an 81 bp RIF-resistance determining region (RRDR).
Methodology: Literatures on RIF resistant mutations published between 2010 and 2016 were thoroughly reviewed.
Results: The most commonly mutated codons in RRDR of *rpoB* gene are 531, 526 and 516. The possibilities of absence of mutation in RRDR of *rpoB* gene in MDR-TB isolates in few studies was due to existence of other rare *rpoB* mutations outside RRDR or different mechanism of rifampicin resistance.
Conclusion: Molecular methods which can identify extensive mutations associated with multiple anti-tuberculous drugs are in urgent need so that the research on drug resistant mutations should be extended.

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Introduction

In 1980s, the duration of treatment of tuberculosis (TB) became shortened from 24 to six months because of the initiation of the short course regimen. However, adherence to treatment regimens was not fully accomplished due to relatively prolonged therapy [1]. Resistance of *Mycobacterium tuberculosis* (*M. tuberculosis*) to a single drug emerged and increased in different parts of the world [1]. In the early 1990s, multiple factors caused an emergence of multiple drug resistant tuberculosis (MDR-TB), known to be resistant to isoniazid and rifampicin which are the two most effective first line anti-tuberculous drugs (anti-TB drugs). In between 2002 and 2006, the MDR-TB was prevalent up to 22% among newly diagnosed cases and up to 60% among previously treated cases [1,2]. In 2002, extensively drug resistant tuberculosis (XDR-TB) was reported in 45 countries, which is defined to be resistant not only to isoniazid and rifampicin but also to at least one fluoroquinolone and to any of the following injectable second-line drugs: kanamycin, amikacin, or capreomycin [2].

Rifampicin (RIF) plays a pivotal role in the treatment of tuberculosis due to its bactericidal effects [3]. Although RIF resistant mutation is difficult to occur when compared with any other anti-TB drugs, the rate of RIF resistance is increasing due to its wide usage [4]. Because the action of RIF is on *rpoB* gene encoding RNA polymerase β subunit, more than 95% of RIF resistant mutations are associated with mutations in *rpoB* gene [5]. The majority of the mutations in *rpoB* gene are present within an 81 bp RIF-resistance determining region (RRDR), a mutation hot spot region. Among the different types of mutations, non-synonymous mutations are more common than insertion, deletions and frameshift mutations [6].

Mutations in *rpoB* gene associated with rifampicin resistance in *M. tuberculosis* isolates of different countries

Two most common mutations in Brazil

Although RIF has fast bactericidal action to tubercle bacilli and has been included in short-course regime, RIF resistance which emerges in the 1990s is a great threat in the control of TB. Mutations in the RRDR of *rpoB* gene contribute over 96% of RIF resistance in *M. tuberculosis* [7]. In the study in Brazil, Ser531Leu and Ser531Trp were the two most common mutations with the 58.5% and 20.8% of RIF resistant isolates [7]. The prevalence of Ser531Leu mutation was nearly the same as other studies in Brazil whereas Ser531Trp mutation was as common as the finding in Chile. The prevalence of this mutation in Chile and Brazil was thought to be a clonal expansion of the LAM9 lineage because this mutation was uncommon in the various regions of the world [7].

Twelve amino acids were surrounding the RIF binding pocket in which RNA polymerase active site is located. Mutation leading to change in one of the amino acids results in modification of active site. Mutation is associated with replacement of an amino acid having a compact side chain by an amino acid with a large side chain [8] and the consequence is inactivation of RNA polymerase resulting in RIF resistance. In the study, two of the isolates harboured the double mutations with one amino acid surrounding RIF binding pocket whereas another amino acid was outside the region of surrounding binding pocket [7]. The fitness cost of the first amino acid was believed to be compensated by the second amino acid. There were two mutations which were outside hotspot region in two isolates [7]. The two single nucleotide polymorphisms (SNPs) were *rpoB* Phe505Val and Ile572Val while Phe505Val was together with Asp516Phe in one isolate. However, two of MDR isolates did not carry any mutation in *rpoB* gene [7].

Multiplex allele-specific polymerase chain reaction in India

Polymerase chain reaction (PCR) followed by DNA-sequencing to find targeted SNP were the preferred method in the identification of RIF resistant mutations and the method was cost-effective and sensitive [7]. Multiplex allele-specific PCR (MAS-PCR) was the method which utilizes three sets of specific primers which were able to detect SNPs at most commonly mutated codons 531, 526 and 516. Seventy to over 90% of RIF resistant isolates harboured mutations in these codons [11].

Earlier detection of *M. tuberculosis* allows implementation of anti-TB treatment in time resulting in earlier control of the infection without wide dissemination [11]. The advantage of a genotypic method for the rapid detection of MDR-TB is faster than conventional methods. MAS-PCR was performed on 90 RIF resistant isolates and 37 RIF sensitive isolates from patients enroled between 2011 and 2013. *rpoB* 516, *rpoB* 526 and *rpoB* 531 mutations in RIF resistant clinical isolates were successfully detected by MAS-PCR assay [12]. Ser531Leu mutation was associated with RIF resistance in 49 isolates whereas His526Tyr SNP and Asp516Val SNP in 17 isolates and 5 isolates respectively [12]. Double mutations were observed in 15 isolates with Ser531Leu and His526Tyr SNPs while triple mutations were observed in 4 isolates with Ser531Leu, His526Tyr and Asp516Val SNPs in MAS-PCR [12].

Mutations outside the 81-bp RRDR have been observed in the study with ten Asn413His, six Asp 435Glu and eight Ala451Asp SNPs whereas mutations within RRDR, which were not able to be detectable by MAS-PCR were Arg511Cys, Val513Asp and Glu521Asp SNPs [12]. Mutations outside the RRDR have rarely been reported with less than 5% of RIF resistant isolates [13]. MIC determination to RIF was done for RIF resistance isolates in the study with the consequence of the finding that seven isolates exhibited high level resistance >128 mg/L. Most of the RIF resistant isolates showed intermediate level of MIC with 40 mg/L [12]. The result in the study was concordant with the finding SNPs at 531 and 526 were the most common mutations observed in other previous studies. Furthermore, MAS-PCR worked well with most common mutations in the *rpoB* RRDR in the study [12].

Mutations at codon no. 490 in Vietnam

In the study, *M. tuberculosis* isolates were collected from TB patients in different regions of Vietnam between 2007 and 2009 [14]. Seventeen different types of mutations were observed in 74 isolates of RIF-resistant *M. tuberculosis* including 57 MDR strains. Single-nucleotide substitutions caused seven amino acid changes in most of the mutations. The common mutations were observed at three codons 531, 526 and 516 as concordant with some studies on RIF resistant markers [14]. Remarkably, occurrence of 4 substitutions at codon 531 was found in the study with Ser/Leu, Ser/Trp, Ser/Phe, Ser/Gln [14]. Similarly mutations at codon 526 were five in number and His/Leu, His/Asn, His/Arg, His/Tyr and His/Ser whereas the change in codon 516 was only one in seven isolates having Asp/Val alteration [14]. Strikingly, the mutation at codon 490 was more common than Asp516Val SNP with two substitutions Gln/His and Gln/Arg [14]. One double mutation was found in the study with Gln490Arg and Ser531Trp [14]. Mutiple mutations at each codon were the reason why there were 17 different mutations in the study although only seven codons harboured mutations. In addition 54 isolates of 57 MDR *M. tuberculosis* carried mutations in 81-bp hypervariable region of *rpoB* gene. Concordant with the other studies, there was no mutation in any RIF susceptible isolates [14].

In RIF-resistant isolates, mutations at *rpoB* codons 531, 526, and 516 are the most common worldwide. However, the frequencies of SNPs in these three codons were variable in different geographic regions [15]. Mutation in codon 531 was the predominant alter-

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