***In silico* mutational analysis in RNA polymerase β subunit (*rpoB*) gene of rifampicin-resistant in *Mycobacterium tuberculosis* from Malaysia**

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Abstract. Tuberculosis (TB) is mainly caused by *Mycobacterium tuberculosis* (MTB) and remains as a key public health problem worldwide. Most of MTB clinical strains are resistant to rifampicin (RIF), the first-line anti-tuberculosis drug. RIF resistance to MTB is due to mutations that mainly found in RIF resistance-determining region (RRDR) in drug target gene, RNA polymerase β subunit (*rpoB*). Therefore, the aim of the study is to extend the identification of variants in *rpoB* gene and to elucidate the effect of variants to the RIF resistance. Four of the strains, MTBR1/09, MTBR2/09, MTBR3/09 and MTB221/11 were subjected to drug susceptibility test (DST). All of the strains sequenced and submitted to the National Center for Biotechnology Information Sequence Read Archive were analyzed to identify the variants in the *rpoB* gene. The identified new variants were then subjected to docking to examine the drug-protein interactions. DST analysis revealed MTBR1/09, MTBR2/09 and MTBR3/09 were resistant to the RIF drug, while MTB221/11 was a presumptive susceptible strain. Two new variants were observed, the deletion (Phe433_Met434delinsLeu in MTBR1/09) and missense (Lys37Arg in MTBR3/09) variants. Meanwhile, the His445Leu, Ser450Leu, Asp103Asp, Ala1075Ala were reported variants. Docking of RIF to MTBR1/09 and MTBR3/09 mutant models revealed the RIF bound to the RIF binding site at different binding affinity and conformation. Concurrently, the new variants caused the RIF to bind to the different active site and neighboring residues. Findings from DST and docking analyses indicate that new variants potentially disturb the RIF inhibition in RpoB mutant proteins, and thus might be responsible to cause the RIF resistance.

Keywords: molecular docking, *Mycobacterium tuberculosis*, rifampicin, RNA polymerase β subunit gene, variant

INTRODUCTION

Tuberculosis (TB) is the ninth leading cause of mortality worldwide. In 2018, an estimated 10 million people have been reported to be infected with TB by World of Health Organization (WHO) (World Health Organization, 2019). The recent enhancement in multi-drug resistant (MDR-TB) and extensively drug resistant (XDR-TB) has worsen the situation. Both situations are

predominantly granted by the accumulation of mutations in drug target genes such as *rpoB*, *pncA*, *katG*, *gyrA* and *gyrB* of MTB (Zhang *et al.*, 2013; Singh *et al.*, 2017).

Mutations in *rpoB* gene can be discovered in 96% of RIF-resistant MTB strains worldwide (Gerald *et al.*, 2014). RIF is one of the important chemotherapeutic agents in the TB treatment

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(Cole, 1996; Campbell *et al.*, 2001; Kumar and Lingaraja, 2014). RIF target is the RNA polymerase (RNAP) enzyme (Zhang *et al.*, 1999; Campbell *et al.*, 2001; Borukhov & Nudler, 2003; Singh *et al.*, 2017). RNAP is an important enzyme in transcription process and it consists of five different subunits, such as α -dimer, β subunit, β' subunit and ω subunit (Zhang *et al.*, 1999; Campbell *et al.*, 2001; Nusrath *et al.*, 2016). RNAP is the last target for regulatory pathways that regulate gene expression in all living organisms (Zhang *et al.*, 1999; Campbell *et al.*, 2001; Nusrath *et al.*, 2016). RIF binds to the β -subunit of the RNAP encoded by the *rpoB* gene and consequently, inhibits the elongation of messenger RNA (Campbell *et al.*, 2001; Almeida da Silva and Palomino, 2011; Singh *et al.*, 2017). In *rpoB* gene, the RIF resistance mostly occurs due to mutation within an 81 base pair (bp) hot spot region or known as RRDR (Almeida da Silva and Palomino, 2011; Pang *et al.*, 2013; Zaw *et al.*, 2018; Al-Mutairi *et al.*, 2019). Previous studies also revealed RIF resistance to the mutations outside of the RRDR (Almeida da Silva and Palomino, 2011; Pang *et al.*, 2013; Singh *et al.*, 2017). Majority of the identified mutations are non-synonymous mutations (Sharma and Madan, 2014; Zaw *et al.*, 2018). However, other changes can also be correlated to the RIF resistance such as insertion, deletion and frameshift (Mani *et al.*, 2001; Sharma and Madan, 2014).

In 2017, there were 26,422 patients in Malaysia diagnosed with TB, with the incidence rate is 81.30/100,000 populations (Fakhruzzaman *et al.*, 2019). In addition, several genome studies have also been carried out to MTB local isolates (Philip *et al.*, 2016; Issa *et al.*, 2016; Fakhruzzaman *et al.*, 2019; Jani *et al.*, 2019). Most of the identified variants from the previous studies are listed in the TB profiler (Fakhruzzaman *et al.*, 2019; Jani *et al.*, 2019). Besides that, *in silico* studies have also been performed to predict the effect of mutations in order to characterize and understand the association between the clinical mutants in RpoB protein and RIF, related to the resistance (Nusrath *et al.*, 2016; Nusrath & Hanna, 2017; Prakash *et al.*, 2017). Therefore, this study was performed to extend the identification of variants spectrum for the most important drug target *rpoB* gene of MTB in Malaysia isolates. Furthermore, structures of the variants along with molecular

docking studies were undertaken to explore the effect these variants to the drug resistance. Finding from this study will provide better understanding to the role of the variants in the mechanism of RIF resistance.

MATERIALS AND METHODS

Identification of variants

The MTBR1/09, MTBR2/09 and MTBR3/09 strains were isolated from pulmonary samples, whereas MTB221/11 was isolated from cerebrospinal fluids (CSF) in Malaysia patients. This study used bacterial isolates, thus, no formal ethics approval was required. Drug susceptibility testing (DST) was performed to all strains with first-line anti-TB drug, namely rifampicin (RIF). All strains have been subjected to whole-genome shotgun sequencing, using Illumina MiSeq platform. The CLC Genome Workbench Software version 6.0.1 was used to filter and analyze the raw sequence data. The sequenced data have also been deposited in the National Center for Biotechnology Information Sequence Read Archive (BioProject ID PRJNA272470) with the first version accession numbers: LFLI000000000 (MTB MTB221/11), LATN000000000 (MTB MTBR1/09), LATO000000000 (MTB MTBR2/09) and LATP000000000 (MTB MTBR3/09).

From the submitted sequences, the drug target gene, *rpoB*, was selected, analyzed and confirmed for their identity using MEGA7 software (version 7), aligning sequences with the MTB reference (Kumar *et al.*, 2016). The nucleotide sequences were converted to amino acids by using Expasy Translate (http://web.expasy.org/cgi-bin/translate/dna_aa) and also referred to the MTB reference using Clustal Omega in order to identify the variants (Sievers *et al.*, 2011). Each variant was compared and validated with that listed in Genome-wide MTB variation (GMTV) database (Chernyaeva *et al.*, 2014) and other available reports of drug resistant MTB genotypes in order to identify either the variant is new or reported.

Molecular modelling of rpoB protein

The three-dimensional (3D) wild type crystal structure of MTB RNA Polymerase beta subunit (RpoB) has been crystallized and available in the Protein Data Bank (PDB) (PDB ID: 5UH6, 5UHB, 5UHC, 5UHD AND 5UHG) (Lin *et al.*, 2017). The crystal structure was visualized using the PyMOL software (version 2.3) (DeLano, 2002). The mutant RpoB protein model was developed according to the user template method using ProMod3 (version 1.3.0), under SWISS-MODEL, a fully automated protein structure homology-modelling server (Waterhouse *et al.*, 2018). The PDB ID: 5UH6 coordinates were used as a template as it contains RNA polymerase (RNAP), nucleic acid scaffold with DNA, three nucleotides of RNA complementary to the template DNA strand and RIF.

The small structure alterations, or clashes occurred during the modelling process were resolved by energy minimization. The server uses CHARMM27 force field for parameterization (Mackerell *et al.*, 2004). QMEAN scoring function was used to evaluate the global and local model quality (Benkert *et al.*, 2011). Good model should present QMEAN Z-scores around zero, which represents the model-built structure and experimental structure in similar size. Meanwhile, scores of -4.0 or below indicates the model structure in low quality (Benkert *et al.*, 2011).

Mutant models validation

The mutant models were then evaluated with ProSA (Wiederstein and Sippl, 2007), which uses the molecular mechanics force field. The overall model quality was predicted by using z-score. PROCHECK (Laskowski *et al.*, 2012) produced the Ramachandran plot statistics for the anticipated model according to stereochemical quality and accuracy, which indicate the acceptance model should have residues located within the accepted regions to be more than 90%. ERRAT (Colovos and Yeates, 1993) was then used mostly to check the quality of non-bonded atomic interactions. The model must have score to be above than 50%, indicating as a good quality model.

Preparation of ligand

For the ligand, the PubChem database was used to retrieve the 3D structure of rifampicin (RIF) (PubChem ID: 135398735) (Bolton *et al.*, 2008). Open Babel was then used to transform the ligand from the SDF format to the PDB format (Guha *et al.*, 2006).

Molecular docking

Initially, redocking was performed in order to confirm the structure and acquire the docking standard parameter. The RIF was redocked to the wild type of RpoB monomer structure. The optimized parameters (exhaustiveness and number of runs) were examined according to the wild type structure to acquire the highest binding affinity and similar RIF conformation.

Initial files for protein and ligand based on PQR and PDB formats, respectively were constructed by AutoDock Tools 1.5.6 (ADT). The two files were transformed to PDBQT, which would be applicable in AutoDock Vina (Sanner, 1999; Trott and Olson, 2010). The grid point spacing of 1.0 Å was used before adjusting the grid box, as that is the criterion unit in AutoDock Vina. The grid box was assigned at 24 Å × 24 Å × 24 Å, centered at the x, y, and z (-12.988, 0.913, 21.035) coordinates, which was to cover all the binding site residues that recognized to interact with the RIF and involved in the catalytic activity; based on the literature (Sekhar *et al.*, 2008). Consequently, docking calculation was performed to both protein and ligand files by AutoDock Vina. The default settings were used for all other parameters. The protein was retained rigid and the ligand was permitted to vary.

The finding was assessed using the binding affinity score in kcal/mol. Classification based on the lowest docked energy would determine the best ligand conformation. The RIF ligands in mutant and wild type structures were overlaid to check the conformation of both ligands. The ligand binding modes, the root mean standard deviation (RMSD) and hydrogen-bond interactions between the protein and the ligand were examined using PyMol (DeLano, 2002; Peterson *et al.*, 2014).

Figure 1. Deletion of c.1299_1301delCAT in MTBR1 strain. (a) Deletion of nucleotide CAT alters the wild type amino acid by deleting two amino acids (Phe and Met). (b) Deletion of amino acids Phe and Met is replaced by only one amino acid, Leu.

p.(Ser450Leu)					
H3Rv	SGLTHKRRL	SALGPGGLSRE	460		
MTB1R	SGLTHKRRL	SALGPGGLSRE	460		
MTB2R	SGLTHKRRL	LALGPGGLSRE	460		
MTB3R	SGLTL KRRL	SALGPGGLSRE	460		
MTB221	SGLTHKRRL	SALGPGGLSRE	460		

p.(Lys37Arg)				p.(His455Leu)			
H3Rv	GAPNRVSFAKL	REPLEVPGLL	48	H3Rv	DQNNPLSGLTH	KRRLSALGP	454
MTB1R	GAPNRVSFAKL	REPLEVPGLL	48	MTB1R	DQNNPLSGLTH	KRRLSALGP	454
MTB2R	GAPNRVSFAKL	REPLEVPGLL	48	MTB2R	DQNNPLSGLTH	KRRLSALGP	454
MTB3R	GAPNRVSFAKL	REPLEVPGLL	48	MTB3R	DQNNPLSGLT	LKRRLSALGP	454
MTB221	GAPNRVSFAKL	REPLEVPGLL	48	MTB221	DQNNPLSGLTH	KRRLSALGP	454

Figure 2. Multiple sequence alignment of *rpoB* gene for four isolates from Malaysia. Changes in amino acids at three positions are shown in blue box.

RESULTS

Variants in *rpoB* gene

The MTBR1/09, MTBR2/09 and MTBR3/09 were phenotype resistant to RIF drug. Meanwhile, one strain, MTB221/11 was a presumptive susceptible strain. Sequencing analyses of *rpoB*

gene were performed for identification of variants. One new deletion variant, three non-synonymous variants and two synonymous variants were identified. A new deletion, c.1299_1301delCAT was identified in MTBR1/09 strain. Deletion of nucleotide CAT caused deletion of two amino acids (Phe and Met), replaced with amino acid, Leu,

(p.(Phe433_Met434delinsLeu) as shown in Figure 1.

Multiple sequence alignment of *rpoB* gene revealed sequence variations in different strains at the amino acid level for three non-synonymous variants (Figure 2). One new missense variant, c.110A>G; p.(Lys37Arg) and one reported missense variant, c.1334A>T; p.(His445Leu) were detected in MTBR3/09 strain. Another missense variant, c.1349C>T; p.(Ser450Leu) was detected in MTBR2/09 strain. Meanwhile, silent variants, c.309C>T; p.(Asp103Asp) was detected in MTB221/09 and c.3225T>C; p.(Ala1075Ala) was identified in MTBR1/09, MTBR2/09 and MTBR3/09. Three variants were located within the RRDR (Phe433_Met434delinsLeu, His445Leu and Ser450Leu), which was near to the RIF binding site, while the other variants located outside the RRDR (Lys37Arg, Asp103Asp and Ala1075Ala).

Mutant modelling

To address the effect of new variants to the RpoB protein structure and to observe how these new variants cause the RIF resistance, homology-modelling server was used to generate the mutant models. The X-ray crystal structure, PDB ID: 5UH6 was used as RpoB wild type reference and template to generate the mutant models. A sequence of MTBR1/09 strain was used to develop a mutant model for Phe433_Met434delinsLeu, while sequence of MTBR3/09 strain was used to develop a mutant model for Lys37Arg and His445Leu. Table 1 shows the two modelled structures were acceptable as all of them have more than 99% similar sequence with the reference template (PDB ID: 5UH6). The two-modelled structures also showed that the QMEAN Z-scores around zero, indicating the model structures and template structure are in similar size.

Validation of the *rpoB* mutant model

The optimized new mutant structures were validated using ProSA-web and PROCHECK programs. ProSA-web was applied to inspect the erroneous of 3D mutant models. The Z-score, which denoted the model quality and random conformation, was used to determine the difference of the total energy of the structure with respect to an energy distribution. The ProSA-web

analysis presented the Z-scores of MTBR1 and MTBR3 mutant models were -14.48 and -14.96 respectively, indicating there was no major deviation from wild type structure of a similar size of the target protein's Z-score, which was -14.59 (Table 1).

PROCHECK was used to further inspect the stereochemistry quality and structure feature. For a better-quality model, the residues positioned in the most favorable and additional allowed regions must be more than 90%. Table 1 shows the scores of wild type and mutant models produced by the PROCHECK were in good quality. The PROCHECK result showed that the 87.6% and 12.2% of wild type residues were located in favored core and in additional allowed regions. Meanwhile, in the mutant models, 92.0% and 7.5% of MTBR1 and, 92.1% and 7.8% of MTB3R residues were located in favored core and in additional allowed regions. As both mutant models showed a good quality, hence, these model structures were used for docking with the RIF inhibitor.

Docking of RIF in *RpoB* wild type and new variant structures

Molecular docking was performed to observe how the new variants cause RIF resistance. The docked complexes were analyzed and PyMOL was used to observe the interactions between RIF and RpoB wild type as well as RpoB mutant proteins. The docking of RIF with RpoB wild type and mutant proteins exhibited the change of binding energies and hydrogen bonds pattern.

Initially, redocking was performed to the wild type structure (RIF and RpoB monomer structure (PDB ID: 5UH6)). The results of redocking infer that the docked of RIF to the RpoB monomer structure bound at the same binding site of wild type X-ray crystal structure. The docked RIF to the RpoB monomer structure produced binding affinity score of -10.2 kcal/mol (Table 2). Superposition of the docked RIF from the wild type structure and RIF from the X-ray crystal structure produced an RMSD of 0.06 Å, yielded a good agreement (Figure 3a). Structural observation showed the docked RIF embedded between 4 active site residues (Gln438, Phe439, Arg454 and Ser456) in the wild type RpoB ligand-binding site, similar to X-ray crystal structure (Table 2). Besides that, the redocked RIF also

formed a new hydrogen bond with Arg173, which previously was not found in the X-ray crystal structure as described in Figure 4a.

Docking of RIF to the MTBR1/09 mutant model produced higher value of binding affinity score of -11.7 kcal/mol than wild type (Table 2). However, superposition between RIF from the mutant model and RIF from wild type structures produced an RMSD of 0.12 Å and exhibited that the deletion of Phe and Met in codon 433 to 434 caused the displacement of RIF (Figure 3b). The RIF was observed to lose hydrogen bond interactions with the active site residues, Arg173, Gln438, Phe439, Arg454 and Ser456 (Figure 4b). Concurrently, the RIF was associated with hydrogen bonds, His1029 (NE2) and Arg67 (NH1) (Figure 4b). This variant also was observed to form a new interaction with neighboring residue, Asn671 (ND2) (Figure 5a).

Meanwhile, docking of RIF to the MTBR3/09 mutant model produced similar binding affinity score (-10.5 kcal/mol) with wild type (Table 2). Superposition between RIF from mutant model and RIF from wild type produced an RMSD of 0.09 Å and exhibited variants, Arg37 and Leu445 to affect the RIF conformation (Figure 3c). However, these variants were observed not to affect the hydrogen bond interactions with all the active site residues (Arg173, Gln438, Phe439, Arg454 and Ser456) (Figure 4c). Concurrently, the new variant, Lys37Arg formed a new hydrogen bond interaction with neighboring residue, Ser540 (O), while reported variant, His445Leu caused loss of hydrogen bond interactions with neighboring residues, Asp435 (N) and Gln432 (OE1) (Figure 5b).

DISCUSSION

The molecular basis of multi-drug resistance in treating tuberculosis has well been examined (Prakash *et al.*, 2017; Fakhruzzaman *et al.*, 2019). Most MTB clinical strains are resistant to one of the first-line drugs, which is RIF (Cole, 1996; Kumar and Lingaraja, 2014; Goldstein, 2014). Earlier studies indicate the major cause of RIF resistance is due to mutations that occur in the most important drug target gene, *rpoB* (Almeida

da Silva and Palomino, 2011). The majority of the mutations are known to alter the nature of the MTB mechanism (Zaw *et al.*, 2018; Al-Mutairi *et al.*, 2019). In the present study, the sequencing analysis of *rpoB* gene from four strains, MTBR1/09 MTBR2/09, MTBR3/09 and MTBR211/09, isolated from Malaysia patients identified the new deletion and missense variants, as well as five reported variants. To the best of our knowledge, the deletion of Phe433_Met434delinsLeu and Lys37Arg identified from MTBR1/09 and MTBR3/09 strains, respectively, were the first variants found in the *rpoB* gene. In addition, the DST revealed the new variants from the two strains were resistant to RIF.

Previous structural studies on the drug resistance of RIF in various mutations of MTB isolate lead to the impairment of the stability and flexibility of RpoB protein binding site (Kumar and Lingaraja, 2014; Prakash *et al.*, 2017). Therefore, docking study was performed to these two new variants in order to investigate how these variants affect the mechanism of RIF resistance. The deletion of Phe433_Met434delinsLeu caused the structural change on MTBR1/09 mutant model due to the deletion of two amino acids and replaced by one amino acid, as compared to the wild type structure. The docking analysis revealed this structural change led to a different orientation and positioning of RIF in the RIF binding site. Concurrently, the higher docking value resulted from the docking of RIF towards the MTBR1/09 mutant model indicates the high capability of RIF to bind to the RIF binding site. However, based on the hydrogen bond interactions analysis and with the consideration that the amino acid deletion occurred close to the active site, RIF was observed to bind to different residues in MTBR1/09 mutant model than the proposed active site residues. As known, hydrogen bonds are important in the structure stability and also in function of biology molecules especially in enzyme catalysis (De Azevedo Jr., 2010). Instead of RIF forming hydrogen bond interactions with 5 active site residues in the wild type structure, the RIF was able to form only 2 hydrogen bonds with the MTBR1/09 mutant model, which might be attributed by the amino acid deletion in the mutant. RIF bound to the MTBR1/09 mutant model with high affinity is probably influenced by

another interaction such as electrostatic force and also stable hydrogen bond. Although the high docking score indicates the high affinity, the deletion changed the nature of RIF binding site

and altered the interactions of RIF with the active site residues, and therefore may contribute to the RIF resistance in this MTBR1/09 mutant protein.

Figure 3. Superimposition of RIF inhibitor in wild type and mutant RpoB structures. (a) Wild type rpoB structure. (b) Deletion of variant, Phe433_Met434delinsLeu in MTBR1/09 strain. (c) Missense variants, Lys37Arg and His445Leu in MTBR3/09 strain. RIF inhibitor in wild type from PDB is in red, RIF inhibitor in wild type from re-docking is in cyan and RIF inhibitor in mutant is in orange. Wild type residues are in yellow. Mutant residues are in purple and magenta.

Table 1. Sequence identity, QMEAN and quality structures of wild type and new mutant models.

Strain	Residue	Sequence identity (%)	QMEAN (Z-score)*	ProSA-web	ProCheck			
				Z-score	Most favoured regions (%)	Additional allowed regions (%)	Generously allowed regions (%)	Disallowed region (%)
Wild Type	NA	NA	NA	-14.59	87.6	12.2	0.2	0.0
MTBR1	Phe433_Met434delinsLeu	99.27	-0.13	-14.48	92.0	7.5	0.3	0.1
MTBR3	Lys37Arg and His445Leu	99.82	-0.26	-14.96	92.1	7.8	0.1	0.0

Note: * Z-scores = ~0, indicating the model structure and template structure are in similar size; Z-scores = -4.0 or below indicates the model structure in low quality.

Table 2. Binding affinity and hydrogen bond interactions between RFP and neighboring residues, between mutant residues and neighboring residues of native and new mutant models.

Strain	Residue	Binding affinity (kcal/mol)	Residue interact with RFP at ligand binding pocket	Wild type residue interact with neighboring residue	Mutant residue interact with neighboring residue
				Wild type residue (Atom): Neighboring residue (Atom)	Mutant residue (Atom): Neighboring residue (Atom)
Wild Type	NA	-10.2	Arg173, Gln438, Phe439, Arg454, Ser456	-NA-	-NA-
MTB1R	Phe433_Met434delinsLeu	-11.7	His1029, Arg607	Met434(N):Glu171(O) Asn679(ND2):Met434(O)	Leu433(O):Asn671(ND2)
MTB3R	Lys37Arg	-10.5	Arg173, Gln438, Phe439, Arg454, Ser456	Lys37(N):Asp972(OD1) Lys37(NZ):Ala538(O)	Arg37(N):Asp972(OD1) Arg37(N):Asp972(OD2) Arg37(NH1):Ser540(O) Arg37(NH2):Ser540(O)
	His445Leu			Asp435(N):His445(ND1) His445(N):Ser441(O) His445(NE2):Gln432(OE1) Arg448(N):His445(O)	Leu445(N):Ser441(O) Leu445(O):Arg448(N)

Figure 4. Hydrogen bond interactions between RIF inhibitor and active site residues in wild type and mutant RpoB structures. (a) Wild type structure. (b) Deletion of variant, Phe433_Met434delinsLeu in MTBR1/09 strain. (c) Missense variants, Lys37Arg and His445Leu in MTBR3/09 strain. RIF inhibitor in wild type and mutant are in cyan and orange, respectively. Wild type residues are in yellow. Mutant residues are in purple and magenta.

Figure 5. Hydrogen bond interactions between wild type, mutant residues, and neighboring residues. (a) Deletion of variant, Phe433_Met434delinsLeu in MTBR1/09 strain. (b) Missense variants, Lys37Arg and His445Leu in MTBR3/09 strain. RIF inhibitor in wild type and mutant are in cyan and orange, respectively. Wild type residues are in yellow. Mutant residues are in purple and magenta.

The effect of the new missense variant, Lys37Arg along with the reported variant, His445Leu on the MTBR3/09 strain causes RIF to bind to the RIF binding site with a different conformation, but still may well preserve the interactions between RIF and RpoB protein. Although the residue of Lys37Arg is located far from the RIF binding site, but not to the residue of His445Leu, which is located near to the RIF binding site. Previous studies revealed the specific variants at residue His445 involve in drug resistance and also can maintain the RIF binding to the RpoB protein (Kumar and Lingaraja, 2014; Miotto *et al.*, 2018). Therefore, based on the outcome, it can be postulated that both variants in MTBR3/09 strain might involve in the RIF resistance.

Previous studies describe majority of the variants are located at RRDR of *rpoB* gene, which spanning codons 433-458 (codon 507-533, *Escherichia coli* numbering system) (Singh *et al.*, 2017; Zaw *et al.*, 2018). About more than 96% of RIF-resistant strains have variants in this region (Almeida da Silva and Palomino, 2011; Kumar and Lingaraja, 2014; Singh *et al.*, 2017; Zaw *et al.*, 2018). The non-synonymous are the more common variants rather than insertion, deletion and frameshift (Zaw *et al.*, 2018). However, several studies reveal that the insertion and deletion variants are also responsible to the RIF resistance (Mani *et al.*, 2001; Sharma and Madan, 2014; Miotto *et al.*, 2018). In conclusion, by considering the location of the new deletion and reported missense variants obtained from this study, the resistance of RIF towards all strains from the DST, and the docking results, it can be postulated that all variants were responsible to cause the RIF resistance. In addition, molecular dynamics simulation is suggested to be carried out to prove the stability of RIF binds to the mutant models and relation with the resistance.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interest.

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