



Mutations in the RpoB Gene of Multidrug-Resistant *Mycobacterium tuberculosis* Isolates from Semarang, Indonesia

Meira Erawati¹, Niken Safitri Dyan Kusumaningrum¹, Megah Andriany¹

*1*Department of Nursing, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia

ARTICLE INFO

Article history:

Received 7 August 2017

Accepted 10 October 2017

Available online 1 December 2017

Keywords:

MDR;

Mycobacterium tuberculosis;

rpoB gene mutations;

rifampicin resistance

ABSTRACT

Tuberculosis is a widespread infectious disease, which is becoming one of the top 10 causes of death worldwide. Mutations of the RpoB gene cause resistance of *Mycobacterium tuberculosis* to rifampicin, which contributes to the occurrence of multidrug-resistant tuberculosis (MDR-TB). The aim of this study was to determine the mutation profile of *rpoB* gene in multi drugs resistant *M. tuberculosis* strains in Semarang, Indonesia. This study employed a cross-sectional design. This study used 24 isolates of *M. tuberculosis* which are MDR-TB. The analysis of the *rpoB* gene mutation in MDR-TB was performed by polymerase chain reaction (PCR) and sequencing method. Most mutations occurred at codon 531 (Ser531Leu)-(13 isolates), codon 490(Gln490Lys)-(7 isolates) and codon 526(5 isolates). Mutational variation mostly occurred at codon 526, with 4 variations of nucleotide changes, including His490Leu, His490Cys, His490Ser and His490Tyr. Thirty missense and one silent mutation were found. The mutations in the *rpoB* gene of MDR *M. tuberculosis* isolates in Semarang showed genetic variations that caused high levels of resistance to rifampicin.

1. Introduction

Most tuberculosis infection in humans is caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) (WHO, 2016). Tuberculosis is a widespread infectious disease, which became one of the top 10 causes of death worldwide in 2015 (WHO, 2016., Syahrini, 2008). There are an estimated 10.4 million cases of tuberculosis, and most of them occur in males of reproductive age. Indonesia has one of the highest rates of tuberculosis infection in the world (Syahrini, 2008). It is predicted that 61,000 deaths per year occur due to tuberculosis infection in this country (Kemenkes, 2014). Another problem caused by tuberculosis infection is the occurrence of multidrug-resistant tuberculosis (MDR-TB), which is predicted to reach 2% of

all new TB cases and 20% of TB cases with repeated treatment. There are an estimated 6,300 cases of MDR-TB each year (Kemenkes, 2014).

Patients are classified having MDR-TB when there is resistance to at least two types of first-line anti-tuberculosis drugs, rifampicin and isoniazid. Rifampicin and isoniazid, are two of the most potent primary drugs used to cure tuberculosis infection (Somasundaram *et al.*, 2014; Lange *et al.*, 2014; Koch *et al.*, 2014). Rifampicin is known as a rapid bactericidal agent against *M. tuberculosis*, and the use of this drug can shorten the care term of tuberculosis infection. Rifampicin almost certainly leads to the occurrence of MDR-TB. For this reason, resistance to rifampicin is often referred to as a

*Corresponding author: Meira Erawati
E-mail address: meiraerawati@gmail.com

surrogate marker of MDR-TB (Raoot and Dev, 2015). Rifampicin acts as an antibacterial against *M. tuberculosis* by directly blocking the process of elongation of ribonucleic acid (RNA) during the beta transcription of a subunit of RNA polymerase (Campbell *et al.*, 2001), which is encoded by the ribonucleic acid of the beta subunit (rpoB) gene (Miller *et al.*, 1994).

Mutations of the rpoB gene are one of the causes of *M. tuberculosis* resistance to rifampicin (Palusch-Oles *et al.*, 2009; Yue *et al.*, 2003). Previous studies in various countries indicated that *M. tuberculosis* isolates were resistant to rifampicin, suggesting mutation in the rpoB gene, particularly in the area of the rifampicin resistance determining region from codon 507 to 533 (Palusch-Oles *et al.*, 2009; Yue *et al.*, 2003; Qazi *et al.*, 2014). The most common form of mutation is the substitution of one amino acid by another, causing changes in the resulting proteins (Yue *et al.*, 2003; Mohajeri *et al.*, 2015; Phelan *et al.*, 2016). The various types of mutation in the rpoB gene, including Ser531Leu and His526Asp, showed a high level of resistance to rifampicin as characterized by the low affinity between the rpoB mutant protein and rifampicin (Pang *et al.*, 2013).

The forms of rpoB gene mutation vary from one strain to another (Mohajeri *et al.*, 2015). One form of mutation in the rpoB gene causes high resistance to rifampicin, whereas other forms of mutations show the opposite response (Mohajeri *et al.*, 2015).

Some countries in the world such as India, Nepal, China, Taiwan, United States of America (USA), and Ethiopia have conducted research on rpoB gene mutation of *M. tuberculosis* (Muthaiah *et al.*, 2017; Yogendra *et al.*, 2017; Daoqun *et al.*, 2017; Lin *et al.*, 2013; Berrada *et al.*, 2016; Tadesse *et al.*, 2016). These studies have shown the discovery of various forms of rpoB gene mutations in *M. tuberculosis*. The nature and frequency of mutations in the rpoB gene in *M. tuberculosis* strains vary considerably with geographical locations or ethnic groups. Information about the mutation of the rpoB gene in *M. tuberculosis* in each country is essential to underpin the search for new drugs that are still sensitive to the bacteria. For that reason, each country particularly countries with high prevalence of MDR TB should have data on the character of the rpoB gene mutation in *M. tuberculosis*. Indonesia is one of the countries

with high incidence of MDR TB, therefore data on rpoB gene mutations in *M. tuberculosis* isolates in Indonesia needs to be well identified. Unfortunately in Indonesia, the data on rpoB gene mutation is still very limited. The primary aim of this study was to determine the mutation profile of rpoB gene in multi drugs resistant *M. tuberculosis* strains in Semarang, Indonesia.

2. Materials and Methods

2.1. Research design

This study employed a cross-sectional design and was conducted from April 2015 to November 2016. The research was conducted in the Health Laboratory of Central Java Province and the Microbiology Laboratory of the Tropical Disease Diagnostic Center (TDDC) at Airlangga University, Surabaya.

2.2. Population

This study used 24 isolates of *M. tuberculosis* which are the collection of the Health Laboratory of Central Java Province. These isolates were collected from April 2015 to May 2016, which were obtained from sputum of tuberculosis patients, of which 9 (34%) were female and 15 (56%) were male. All patients were adults over 15 years of age.

The bacteria used in this study met the inclusion criteria, which are included in the MDR TB category. These isolates had been tested for their resistance to the first-line anti-tuberculosis drugs, including rifampicin, isoniazid, streptomycin and ethambutol. The isolates were classified as MDR-TB when they were proven to be resistant to at least two types of anti-tuberculosis drugs: rifampicin and isoniazid. The isolates were excluded from the study when the rpoB gene product is not the same as the control.

2.3. Ethical consideration

This study received approval from the Ethics Committee of the Faculty of Medicine of Sultan Agung Islamic University, Semarang.

2.4. Data collection

Anti-tuberculosis drug resistance testing

Resistance to first-line anti-tuberculosis drugs was tested in the Health Laboratory of Central Java. The method used was indirect proportional modification with the culture medium of Lowenstein-Jensen (LJ). Each sample was planted in a series on LJ medium containing the first-line anti-tuberculosis drugs, namely streptomycin, isoniazid, rifampicin, and ethambutol. The series of tubes which had been inoculated with the bacterium was then incubated at a temperature of 37°C for 3 weeks, and changes in growth were observed every week. In the control medium, the bacteria would grow confluent and this was considered 100% growth. To determine the resistance, a comparison between the percentage of bacterial growth on the drug and control media was carried out. When the number of colonies of bacteria that grew on a medium containing anti-tuberculosis drugs was more than 1%, then the bacteria was considered resistant.

2.5. DNA was isolated with a DNeasy Mini spin column following the manufacturer's instructions

DNA isolation has been performed using the Qiagen kit DNeasy Blood and Easy kit with catalog number 69504. The procedure works as follows: PBS was added with a pipette to a volume of 220µl. A 1.5-ml tube was inoculated with a loopful of bacteria. Twenty microliters of proteinase K was combined with 200µl Buffer AL, and vortexed to mix. Then, 200µl ethanol (96%-100%) was added and mixed again by vortexing. The mixture was a DNeasy Mini spin column and then in a 2ml tube, and centrifuged at 8000 rpm for 1 min. The lower spin column and its contents were removed and replaced with another spin column. A 500-µl volume of buffer AW1 was added and then centrifuged at 8000 rpm for 1 min. The lower spin column and its contents were removed again and replaced with a new spin column. A 500µl volume of buffer AW2 was added and centrifuged at a speed of 14,000 rpm for 3 min. The lower spin column and its contents were removed, and the column

was placed on a 1.5-ml microcentrifuge tube and its content were removed and the column was moved on microcentrifuge tube. The DNA was eluted with 50µl buffer AE placed in the middle of the membrane, then incubated at room temperature for 1 min, and centrifuged at 8000 rpm for 1 min.

2.6. PCR and sequencing

The DNA template was amplified in a final reaction of 50 µl, consisting of 20 µl PCR mix, 1µl forward primer, 1 µl reverse primer, 3 µl DNA, 20 µl D_w. A pair of primer (forward, 5'-TAC GGT CGG CGA GCT GAT CC-3'; reverse, 5'-TAC GGC GTT TCG ATG AAC C-3'), targeting a 411-bp fragment of the *rpoB* gene, was used with the following PCR cycling program: 94°C for 2 min; 35 cycles of denaturation at 94°C for 20 s, annealing at 61,3°C for 10 s, and extension at 72°C for 30 s; and a final extension at 72°C for 5 min, and elongation at 4°C until completion.

The amplified product was separated on 1.5% agarose gel and stained with ethidium bromide followed by visualization under ultraviolet light in a gel documentation system. The result of visualization of the *rpoB* gene is shown in Figure 1. The PCR products were sequenced using automated DNA sequencers (Applied Biosystems, Inc., Foster City, Calif) and analyzed using the BLAST bioinformatics tools available at the National Center for Biotechnology Information compared with wild-type *M. tuberculosis* (H37Rv). The process of DNA isolation, PCR, and sequencing were performed in the Laboratory of Microbiology, TDDC in Airlangga University, Surabaya.

2.7. Statistical analysis

The data were collected and analyzed by using the Microsoft Excel program. For a clear presentation, all the results were presented in tables and diagrams.

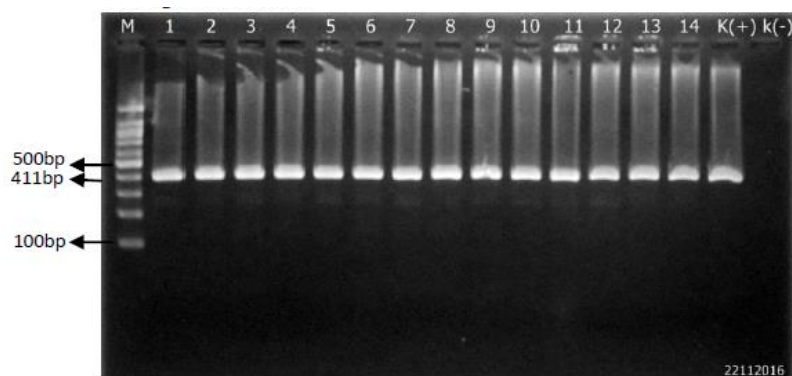


Figure 1. The PCR products of *rpoB* gene of MDR *M. tuberculosis*. M: marker ladder of 100 bp; Number 1 to 14: 1-14 isolates of DNA *M. tuberculosis*; K (+): positive control; k (-): negative control. The size of PCR product of *rpoB* gene of *M. tuberculosis* is 411 bp.

3. Results

A resistance test to first-line anti-tuberculosis drugs (rifampicin, isoniazid, streptomycin and ethambutol) was performed on all samples used in the study (24 isolates). All isolates were in the MDR-TB category. The results of the resistance test of all samples are shown in Table 1.

PCR and sequencing were performed on the DNA of 24 isolates of MDR *M. tuberculosis* to identify the presence of mutations in the *rpoB* gene. The results of sequencing of the 24 isolates indicated single-base substitutions along the 411- bp fragment of the *rpoB* gene.

Mutations were found in 22 isolates with the highest number of mutations at codon 531. The highest mutational diversity occurred at codon 526, with 4 different base substitutions: $\underline{C}AC \rightarrow \underline{C}TC$, $\underline{C}AC \rightarrow \underline{T}GC$, $\underline{C}AC \rightarrow \underline{T}CC$, and $\underline{C}AC \rightarrow \underline{T}AC$. In all isolates, the types of mutations were missense (30 mutations) and silent (one mutation). Double mutations occurred in 7 isolates, including isolate numbers 534, 589, 591, 593, 930, 984, and 1239. Changes in the nucleotide and protein in the *rpoB* gene can be seen in Table 2.

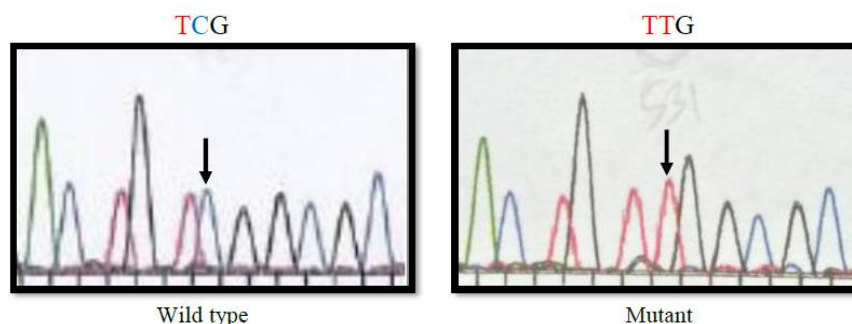


Figure2. Mutation in *rpoB* gene TCG \rightarrow TTG in codon 531

Table 1. The pattern strains resistance of *M. tuberculosis* to first-line anti-tuberculosis drugs (n=24)

Drugs	Number of resistant strain	(%)
Rif+Inh	2	8.3
Rif+Inh+Sm	3	12.5
Rif+Inh+Emb	8	33.3
Rif+Inh+Sm+Emb	11	45.8

Rif-Rifampicin, Inh-Isoniazid, Sm-Streptomycin, Emb-Ethambutol,

Table 2. The form of mutations and protein changes found in the *rpoB* gene of the isolates of MDR *M. tuberculosis*

Mutated codon	Nucleotide change	Amino acid change	Type of mutation	Number of isolates	Sample ID
490	<u>C</u> AA→ <u>A</u> AA	Gln→Lys	Missense	7	534, 589, 591, 846, 930, 984, 1239
511	<u>C</u> TG→ <u>C</u> GG	Leu→Arg	Missense	1	1239
513	<u>C</u> AA→ <u>G</u> AA	Gln→Glu	Missense	1	779
	<u>C</u> AA→ <u>C</u> CA	Gln→Pro	Missense	1	1282
516	<u>G</u> AC→ <u>G</u> TC	Asp→Val	Missense	1	589
519	<u>A</u> AC→ <u>A</u> AT	Asn→Asn	Silent	1	1336
526	<u>C</u> AC→ <u>C</u> TC	His→Leu	Missense	1	593
	<u>C</u> AC→ <u>T</u> GC	His→Cys	Missense	1	846
	<u>C</u> AC→ <u>T</u> CC	His→Ser	Missense	2	986, 1239
	<u>C</u> AC→ <u>T</u> AC	His→Tyr	Missense	1	1242
531	<u>T</u> CG→ <u>T</u> TG	Ser→Leu	Missense	12	534, 579, 591, 930, 985, 1072, 1102, 1104, 1154, 1241, 1283, 1284
	<u>T</u> CG→ <u>C</u> TG	Ser→Leu	Missense	1	984
535	<u>C</u> CC→ <u>C</u> AC	Pro→His	Missense	1	593

Arg-Arginine, **Lys**-Lysine, **Leu**-Leucine, **Gln**-Glutamine, **Glu**-Glutamic acid, **Pro**-Proline, **Asp**-Aspartic acid, **Val**-Valine, **Asn**-Asparagine, **His**-Histidine, **Cys**-Cysteine, **Ser**-Serine, **Tyr**-Tyrosine, **Trp**-Tryptophan

4. Discussion

In this study, mutations in the *rpoB* gene of *M. tuberculosis* could be found in 22 (91.7%) isolates at different locations, while in the other 2 (8.3%) isolates, no mutations were identified. Several previous studies reported that the frequency of mutations in the *rpoB* gene of *M. tuberculosis* isolates resistant to rifampicin, either single or with other anti-tuberculosis drugs (Wang *et al.*, 2013), could reach more than 90% (17-19), or even 100% (Horng *et al.*, 2015).

This study identified 13 patterns of mutations that caused changes in the sequence of bases in some codons along the *rpoB* gene. Most mutations were found at codon 531 (13 isolates), codon 490 (7 isolates), and codon 526 (5 isolates). The positions of the mutated codon in

the *rpoB* gene were proven to determine the level of resistance of *M. tuberculosis* isolates to rifampicin. Mutation at codons 523, 526, and 531 proved to be the cause of a high level of resistance to rifampicin, which was found in various regions of the world (Yue *et al.*, 2003; Sharma, 2014). Meanwhile, mutation at codons 510, 512, and 515 showed a low level of resistance to rifampicin, while the mutation at codon 513 did not cause any resistance to rifampicin (Zaczek *et al.*, 2009). Thus, the high frequency of mutations involving codons 531 and 526 in this study showed that the majority of MDR isolates of *M. tuberculosis* found in Semarang indicated a high level of resistance to rifampicin.

The level of resistance of *M. tuberculosis* to rifampicin is also determined by the presence of a double mutation and protein changes that

occur in certain codons. A single mutation that occurred at codon 516 (D/V) showed a high level of resistance to rifampicin, whereas a single mutation in 516 (D/Y) and a double mutation at a similar codon, namely codon 516 (D/G), caused a low level of rifampicin resistant (Zaczek *et al.*, 2009). In contrast with previous studies, the mutation at codon 516 (D/H) did not cause rifampicin resistant (Nakata *et al.*, 2012). Compared with previous studies, the present study also found a single mutation at codon 516 (D/V) of MDR *M. tuberculosis* isolate from Semarang which indicated a high level of rifampicin resistant.

In this study, 7 isolates had mutations at codon 490. These codons were outside of the “hot spot” area of *rpoB* gene. Mutations in this codon are often found in rifampicin resistant isolates of *M. tuberculosis* (Minh *et al.*, 2012). Mutation at codon 490 is associated with a particular strain of bacteria. In a previous study, a mutation at codon 490 of *M. tuberculosis* was found only in Beijing and Central Asia, and was not found in the samples of Harleem and East African-Indian (Doustdar *et al.*, 2008). The *M. tuberculosis* isolates identified in two major cities in Indonesia, namely Jakarta and Bandung were largely the Beijing strains, which were notoriously difficult to treat (Parwati *et al.*, 2010). The present study identified mutations at codon 490 in *M. tuberculosis* isolates in Semarang city in a significant number (7 isolates), which most likely showed the Beijing strain. However, further study is needed to identify the micobacterial type.

One factor that determines *M. tuberculosis* isolate resistance to rifampicin is protein changes due to mutations in certain codons. The *RpoB* gene nucleotide changes in this study were entirely due to the substitution of one nucleotide by another. In addition, changes in the composition of the nucleotides in the *rpoB* gene could also occur due to deletions or insertions (Sharma, 2014 and Fan *et al.*, 2003). However, the substitution process was the most common cause of nucleotide changes in the *rpoB* gene (Ramasoota *et al.*, 2006). The *RpoB* gene mutations of *M. tuberculosis* isolates at codon 531, may cause the serine change to leucine. It usually occurred in isolates with high-level resistance to rifampicin (Ramasoota *et al.*, 2006). Another mutation at codon 531 was found in the form of changes in the nucleotide

composition of the TCG (Ser)→TTC (Phe) (Nakata, 2012), although these mutations were scarce. In this study, there were 13 isolates which had mutations at codon Ser531Leu. This was the reason why the *rpoB* mutations at codon 531 of MDR *M. tuberculosis* isolates from Semarang had high levels of resistance to rifampicin.

The mutation at codon 526 showed the highest variation in *rpoB* gene nucleotide changes. These changes included CAC (His) → CCC (Pro), CAC (His) → CGC (Arg), CAC (His) → CTC (Leu), CAC (His) → GAC (Asp), CAC (His) → TAC (Tyr) (Yue *et al.*, 2003., Ma *et al.*, 2006., Paluch-Oles *et al.*, 2009). When the *rpoB* mutation of *M. tuberculosis* isolates occurs at codon 526, it would cause a high-level of resistance to rifampicin (Mohajeri *et al.*, 2015). In the present study, involving the MDR *M. tuberculosis* isolates, the highest mutation variation also occurred at codon 526. Four different mutations have been found in this study. All isolates also showed a high resistance to rifampicin.

This research has several advantages. First of all, this study was a pilot study conducted in Semarang, Central Java Province. Similar research has not been done in Semarang and surrounding areas. Second, the results of this study were very useful for the development of further research. Third, this research is supported by a laboratory that has gained recognition from the Ministry of Research and Technology of the Republic of Indonesia.

Although this research was carefully prepared, it was still having weakness. The population of the study is small, only twenty four isolates and need to add more samples for better results.

Conclusion

This study showed that the MDR *M. tuberculosis* isolates from Semarang had high levels of resistance to rifampicin. The *rpoB* gene factors affecting the high levels of resistance of isolates to rifampicin included the mutated codon location, the forms of mutation, and the variation in mutations that occurred in certain codons.

Although this research has reached its aims, there was an unavoidable limitation. This research was conducted only on samples which

were taken from Semarang and its surroundings. It might not represent the majority of the rpoB gene mutation pattern of MDR TB in Indonesia. Therefore, to generalize the results for larger groups, the study should have involved more samples from different places in Indonesia.

Acknowledgments

The authors would like to express their acknowledgment to Agnes Dwi Sis Perwitasari for her technical assistance with molecular analysis. Furthermore, the authors also express their gratitude to Diponegoro University for the research funding of this study in 2016.

Conflict of Interest

None of the authors of this paper have a conflict of interest.

References

- Anonymous. (2011) Stop TB terobosan menuju akses universal strategi nasional pengendalian Tb di Indonesia 2010-2014. Jakarta. Direktorat Jenderal Pengendalian Penyakit dan Pengobatan Lingkungan Kementerian Kesehatan Republik Indonesia.
- Bahrmand, A., Titov, L., Tasbiti, A., et al. (2009) High-Level Rifampin Resistance Correlates with Multiple Mutations in the rpoB Gene of Pulmonary Tuberculosis Isolates from the Afghanistan Border of Iran. *J. Clin. Microbiol.* 47(9): 2744–2750.
- Berrada, Z., Lin, S., Rodwell, T., et al. (2016) Rifabutin and rifampin resistance levels and associated rpoB mutations in clinical isolates of *Mycobacterium tuberculosis* complex. *Diagn. Microbiol. Infect. Dis.* 85:177–181.
- Campbell, E., Korzhewa, N., Mustaev, A., et al. (2001) Structural Mechanism for Rifampicin Inhibition of Bacterial RNA Polymerase. *Cell.* 104:901–912.
- Daoqun, L., Yuzhu, S., Cheng-Lin, X., et al. (2017) Screening mutations in drug-resistant *Mycobacterium tuberculosis* strains in Yunnan, China. *J. Infect. Public.* 10:630–636.
- Doustdar, F., Khosravi, A., Farnia, P., et al. (2008) Mutations in rpoB Gene and Genotypes of Rifampin Resistant *Mycobacterium tuberculosis* Isolates in Iran. *Tanaffos.* 7(2): 11–17.
- Fan, X., Fan, X., Hu, Z., et al. (2003) Rapid Detection of. *Microbiology. J. Clin. Microbiol.* 41(3):993–997.
- Garcia, L., Alonso-Sanz, M., Rebollo, M., et al. (2001) Mutations in the rpoB Gene of Rifampin-Resistant *Mycobacterium tuberculosis* Isolates in Spain and Their Rapid Detection by PCR–Enzyme-Linked Immunosorbent Assay. *J. Clin. Microbiol.* 39(5):1813–1818.
- Hirano, K., Abe, C., Takahashi, M. (1999) Mutations in the rpoB Gene of Rifampin-Resistant *Mycobacterium tuberculosis* Strains Isolated Mostly in Asian Countries and Their Rapid Detection by Line Probe Assay. *J. Clin. Microbiol.* 37(8): 2663–2666.
- Horng, Y., Jeng, W., Chen, Y., et al. (2015) Molecular analysis of codon 548 in the rpoB gene involved in *Mycobacterium tuberculosis* resistance to rifampin. *Antimicrob. Agents. Chemother.* 59:1542–1548.
- Koch, A., Mizrahi, V., Warner, D. (2014) The impact of drug resistance on *Mycobacterium tuberculosis* physiology: what can we learn from rifampicin?. *Emerg. Microbes. Infect.* 3(3):1–11.
- Lange, C., Abubakar, I., Alffenaar, J., et al. (2014) Management of patients with multidrug-resistant/ extensively drug-resistant tuberculosis in Europe: A TBNET consensus statement. *Eur. Respir. J.* 44(1):23–63.
- Lin, Y., Tai, C., Li, C., Lin, C. (2013) Resistance profiles and rpoB gene mutations of *Mycobacterium tuberculosis* isolates in Taiwan. *J. Microbiol. Immunol. Infect.* 46:266–270.
- Ma, X., Wang, H., Deng, Y., et al. (2006) rpoB Gene Mutations and Molecular Characterization of Rifampin-Resistant *Mycobacterium tuberculosis* Isolates from Shandong Province, China. *J. Clin. Microbiol.* 44(9): 3409–3412.
- Makadia, J., Jain, A., Patra, S., et al. (2012) Emerging trend of mutation profile of rpoB gene in MDR tuberculosis, North India. *Indian. J. Clin. Biochem.* 27(4):370–374.
- Miller, L., Crawford, J., Shinnick, T. (1994) The rpoB gene of *Mycobacterium tuberculosis*. *Antimicrob. agents.* 38(4):805–811.
- Minh, N., Bac, N., Son, N., et al. (2012) Molecular Characteristics of Rifampin- and Isoniazid-Resistant *Mycobacterium tuberculosis* Strains Isolated in Vietnam. *J. Clin. Microbiol.* 50(3): 598–601.
- Mohajeri, P., Sadri, H., Farahani, A., et al. (2015) Norozi B, Atashi S. Frequency of Mutations Associated with Rifampicin Resistance in *Mycobacterium tuberculosis* Strains Isolated from Patients in West of Iran. *Microb. Drug. Resist.* 21(3):315–319.
- Muthaiah, M., Shivekar, S., Kapalamurthy, V., et al. (2017) Prevalence of mutations in genes

- associated with rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates. *J. Clin. Tuberc. Other. Mycobact. Dis.* 8:19–25.
- Nakata, N., Kai, M., Makino, M. (2012) Mutation Analysis of *Mycobacterial rpoB* Genes and Rifampin Resistance Using Recombinant *Mycobacterium smegmatis*. *Antimicrob. Agents. Chemother.* 56(4): 2008–2013.
- Paluch-Oleś, J., Koziol-Montewka, M., Magrys, A. (2009) Mutations in the *rpoB* gene of rifampin-resistant *Mycobacterium tuberculosis* isolates from Eastern Poland. *New. Microbiol.* 32(2):147–152.
- Pang, Y., Lu, J., Wang, Y., et al. (2013) Study of the rifampin monoresistance mechanism in *Mycobacterium tuberculosis*. *Antimicrob. Agents. Chemother.* 57(2):893–900.
- Parwati, I., Alisjahbana, B., Apriani, L., et al (2010) *Mycobacterium tuberculosis* Beijing genotype is an independent risk factor for tuberculosis treatment failure in Indonesia. *J. Infect. Dis.* 201(4):553–557.
- Phelan, J., Coll, F., McNerney, R., et al. (2016) *Mycobacterium tuberculosis* whole genome sequencing and protein structure modelling provides insights into anti-tuberculosis drug resistance. *BMC. Med.* 14(31):1–13.
- Prim, R., Schörner, M., Senna, S., et al. (2015) Molecular profiling of drug resistant isolates of *Mycobacterium tuberculosis* in the state of Santa Catarina, southern Brazil. *Mem. Inst. Oswaldo. Cruz.* 110(5):618–623.
- Qazi, O., Rahman, H., Tahir, Z., et al. (2014) Mutation pattern in rifampicin resistance determining region of *rpoB* gene in multidrug-resistant *Mycobacterium tuberculosis* isolates from Pakistan. *Int. J. Mycobacteriology.* 3(3):173–177.
- Ramasoota, P., Pitaksajakul, P., Phatihattakorn, W. (2006) Mutation in the *rpoB* gene of rifampicin-resistant *Mycobacterium tuberculosis* strains from Thailand and its evolutionary implication. *Southeast. Asian. J. Trop. Med. Public. Health.* 37(1):136-147.
- Raoot, A., Dev, G. (2015) Evaluate “Rifampicin Resistance” as Surrogate Marker for Rapid Detection of MDR-TB Using Real-Time PCR Directly on FNAC Samples of Tuberculous Lymphadenitis. *Br. J. Med. Med. Res.* 9(5): 1–8.
- Sharma, S. (2014) Detection of Mutations in *rpoB* Gene of Clinically Isolated *M. Tuberculosis* by DNA Sequencing. *Mycobact. Dis.* 4(4):1–5.
- Somasundaram, S., Ram, A., Sankaranarayanan, L. (2014) Isoniazid and Rifampicin as Therapeutic Regimen in the Current Era: A Review. *J. Tuberc. Res.* 02:40–51.
- Tadesse, M., Aragaw, D., Dimah, B., et al. (2016) Drug resistance-conferring mutations in *Mycobacterium tuberculosis* from pulmonary tuberculosis patients in Southwest Ethiopia. *Int. J. Mycobacteriol.* 5:185–191.
- Wang, S., Zhao, B., Song, Y., et al. (2013) Molecular characterization of the *rpoB* gene mutations of *Mycobacterium tuberculosis* isolated from China. *J. Tuberc. Res.* 1(1):1–8.
- World Health Organization. Global tuberculosis report 2016. World Health Organization Document 2016. WHO/HTM/TB/2016. 13:1–120. GoogleScholar
- Yogendra, S., Bhagwan, M., Jeewan, T., et al. (2017) High diversity of multidrug resistant *Mycobacterium tuberculosis* Central Asian Strain isolates in Nepal. *J. Infect. Dis.* S1201-9712(17)30164-169.
- Yue, J., Shi, W., Xie, J., et al. (2003) Mutations in the *rpoB* Gene of Multidrug-Resistant *Mycobacterium tuberculosis* Isolates from China. *J. Clin. Microbiol.* 41(5): 2209–2212.
- Zaczek, A., Brzostek, A., Augustynowicz-Kopec, E., et al. (2009) Genetic evaluation of relationship between mutations in *rpoB* and resistance of *Mycobacterium tuberculosis* to rifampin. *BMC. Microbiol.* 9(10):1-8.