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ORIGINAL PAPER

# Novel mutations detected from drug resistant *Mycobacterium tuberculosis* isolated from North East of Thailand

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## Abstract

The emergence of drug-resistant tuberculosis is a major global public health threat. Thailand is one of the top 14 countries with high tuberculosis and multi-drug resistant tuberculosis rates. Immediate detection of drug-resistant tuberculosis is necessary to reduce mortality and morbidity by effectively providing treatment to ameliorate the formation of resistant strains. Limited data exist of mutation profiles in Northeastern Thailand. Here, 65 drug-resistant *Mycobacterium tuberculosis* isolates were used to detect mutations by polymerase chain reaction (PCR) and DNA sequencing. In the *katG* gene, mutations were occurred in 47 (79.7%) among 59 isoniazid resistant samples. For *rpoB* gene, 31 (96.9%) were observed as mutations in 32 rifampicin resistant isolates. Of 47 *katG* mutation samples, 45 (95.7%) had mutations in *katG*315 codon and 2 (4.3%) showed novel mutations at *katG*365 with amino acid substitution of CCG-CGG (Pro-Arg). Moreover, out of 31 *rpoB* mutation isolates, the codon positions *rpoB*516, *rpoB*526, *rpoB*531 and *rpoB*533 were 3 (9.7%), 8 (25.8%), 11 (35.5%) and 1 (3.2%), respectively. Seven isolates of double point mutation were found [*rpoB*516, 526; 1 (3.2%) and *rpoB*516, 531; 6 (19.4%)]. In addition, 1 (3.2%) sample had triple point mutation at codon positions *rpoB*516, 526 and 531. Common and novel mutation codons of the *rpoB* and *katG* genes were generated. Although DNA sequencing showed high accuracy, conventional PCR could be applied as an initial marker for screening drug-resistant *Mycobacterium tuberculosis* isolates in limit resources region. Mutations reported here should be considered when developing new molecular diagnostic methods for implementation in Northeastern Thailand.

**Keywords** Drug-resistant · *Mycobacterium tuberculosis* · Mutation · *rpoB* · *katG*

## Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) bacteria is a serious global infectious disease even though diagnosis and treatment are available. About one fourth of population (10 million people) in the world is suffering from this disease, with death rates of 1.4 million people in 2019 (World Health Organization 2020). The emergence of multi-drug resistant TB (MDR TB), described as resistant to at least rifampicin and isoniazid, as the two most commonly applied powerful first-line anti-TB drugs, is also a major public health threat worldwide (Lange et al. 2019; Jang and Chung 2020). Globally in 2019, an estimated 465,000 incident cases were reported as MDR and rifampicin resistant (RR) TB, while 3.3% and 13% of new cases and 18% and 17.4% of previously treated cases were diagnosed with MDR/RR TB and isoniazid (INH) resistant TB, respectively (World Health Organization 2020). Thailand is one of the top 14 countries with high TB, TB/HIV and MDR-TB rates and had 150 TB cases per

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100,000 population in 2019 (World Health Organization 2020; Anukool et al. 2020). Immediate detection and accurate identification of MDR-TB is necessary to reduce mortality and morbidity by effectively providing treatment, to ameliorate the formation and spread of resistant strains (Jaksuwan et al. 2017; Nimri et al. 2011; Prammananan et al. 2008). The phenotypic DST method is the gold standard for detection of drug resistant but this takes 2–3 months to yield results and also increases the risk of bacterial contamination and cross infection by non-tuberculous mycobacterial infection. To shorten the turnaround time, two rapid molecular diagnostic methods, polymerase chain reaction (PCR) and DNA sequencing were selected in this study (Genestet et al. 2020; Li et al. 2020; Jang and Chung 2020). The distribution of drug-resistant genes varies according to geographical locations and identifying the mutation patterns of MDR-TB is important to optimize the treatment protocol (Jaksuwan et al. 2017; Seifert et al. 2015). However, limited data exist of mutation profiles in Northeastern Thailand.

A better understanding of drug-resistant mechanisms and the most commonly found mutation codons of rifampicin (RIF) and isoniazid (INH) are required. RIF is one of the most effective anti-TB drugs. Its mode of action involves binding to the β-subunit of RNA polymerase, thereby inhibiting the elongation of messenger RNA (Palomino and Martin 2014). The mechanism of RIF resistance in MTB is associated with mutation of the *rpoB* gene (Isakova et al. 2018). Some 96% of the mutation codons of RIF resistant MTB isolates mostly occur in 81 bp spanning codons (507–533), called the rifampicin resistance determining region (RRDR). Among these codons, *rpoB*516, 526 and 531 provided the highest level of resistance with percentage of 70–95% (Palomino and Martin 2014; Tajbakhsh et al. 2018). Another powerful drug for MTB is isoniazid and mutations in several genes such as *katG*, *inhA*, *oxyR*, *kasA*, *ahpC* and *ndh* are responsible for INH drug resistance. Most INH resistance is caused by mutation in the *katG* gene that codes for the catalase-peroxidase enzyme, a key factor in mycolic acid biosynthesis. The most common mutation in INH-resistant strains occurs at the codon position 315 of *katG* gene (*katG*315) reported that on average 50–95% (Gupta et al. 2013; Pandey et al. 2017; Ravibalan et al. 2015).

Here, we examined the mutations of drug-resistant MTB strains emphasizing on the *rpoB* and *katG* genes in Northeastern Thailand populations by the used of PCR and DNA sequencing methods.

## Materials and methods

### Sample sources and processing

A total of 357 MTB isolates were randomly selected from the TB laboratory at the Office of Disease Prevention and Control Center (DPC) 7th, 8th, 9th and 10th (Khon Kaen Province, Udon Thani Province, Nakhon Ratchasima Province and Ubon Ratchathani Province, respectively) between 2013 and 2016 in Northeastern Thailand. Among these samples, 92 isolates were found with drug resistance (rifampicin and isoniazid) by testing with the standard proportion method in all DPC centers (Rueangsak et al. 2020). In this study, 65 drug-resistant MTB isolates were used for detection of mutation patterns in Northeastern Thailand. All isolates were confirmed as MTB by observing culture positive on Lowenstein-Jensen medium and using immunochromatographic assay (ICA) kit (SD Bioline TB Ag MPT64 rapid test) to detect and amplify real-time PCR.

### DNA extraction

Genomic DNA of the 65 drug-resistant isolates of *Mycobacterium tuberculosis* (26 resistant to both RIF and INH, 33 mono-resistant to isoniazid and 6 mono- resistant to rifampin) were extracted using the CTAB (cetyl trimethylammonium bromide) method (Van Embden et al. 1993). In two microcentrifuge tubes, each 400 µL of bacterial cell suspension was transferred. After that, both tubes were put on heat block with the temperature at 80 °C for 20 min. After adding all chemical reagents, the DNA pellets were re-dissolved in 50 µL of TE buffer and stored at -20 °C and -70 °C until required for further use. The estimation of DNA concentration was done by using a spectrophotometer (NanoDrop 2000c, USA) with the absorbance at a wavelength of 260 nm.

### PCR amplification

Conventional polymerase chain reaction was used to determine mutations in 4 regions for the *rpoB* gene codons 516, 526, 531 and 533 and 1 region for the *katG* gene codon 315 using specific primers (Table 1) (Nurpermatasari et al. 2018; Ahmed et al. 2013). For each condition, the preparation of master mix concentration and cycling temperature was modified from the methods of (Allegui et al. 2012; Tajbakhsh et al. 2018; Farnia et al. 2010).

Amplification reactions for the *rpoB*516 and *katG*315 codons were performed in a final volume of 50 µL containing 0.5 µL of 2.5 mM dNTPs, 5 µL of 10X Buffer, 0.25 µL of 5 U/ µL Taq polymerase, 1.66 µL of 50 mM $MgCl_2$, 40.59 µL sterile DI water, 0.5 µL of each forward and reverse 10 µM primers and 1 µL of 20 ng genomic DNA. Similar concentrations of all reagents were also used for amplifying the *rpoB*531 and *rpoB*526 codons with a final volume of 25 µL composed of 0.25 µL of dNTPs, 2.5 µL of Buffer, 0.2 µL of Taq polymerase, 0.5 µL (for 531 codon) and 1 µL (for 526 codon) of $MgCl_2$, 18.55 µL and 18.05 µL of sterile DI water for 531 and 526 codons, respectively. After 1 µL of each

**Table 1** Nucleotide sequences of the PCR primers used for detection of *rpoB* and *katG*315 genes

| Gene name with codon position | Primers sequences (5´ 3´) | Product size (bp) | References |
|---|---|---|---|
| *rpoB*531 | CACAAGCGCCGACTGTC<br>TTGACCCGCGCGTACAC | 170 | Nurpermatasari et al. (2018) |
| *rpoB*526 | CTGTCGGGGTTGACCCA<br>TTGACCCGCGCGTACAC | 185 | Nurpermatasari et al. (2018) |
| *rpoB*516 | CAGCTGAGCCAATTCATGGA<br>TTGACCCGCGCGTACAC | 218 | Nurpermatasari et al. (2018) |
| *rpoB*533 | CGCCGACTGTCGGCGCT<br>TTGACCCGCGCGTACAC | 163 | Nurpermatasari et al. (2018) |
| *katG*315 | GCAGATGGGGCTGATCTACG<br>ATACGACCTCGATGCCGC | 292 | Ahmed et al. (2013) |

primer and 1 µL of DNA. For the *rpoB*533 amplification, the PCR reaction was performed in a final volume of 25 µL containing the same concentrations of the above reagents accompanied by dNTPs (2 µL), Buffer (2.5 µL), Taq (0.25 µL), $MgCl_2$ (2.75 µL), DI water (13 µL), forward primer (0.5 µL), reverse primer (2 µL) and DNA (2 µL).

The DNA amplifications were performed in a thermal cycler, FlexCycler2, (Analytik Jena AG, Germany). Cycling temperatures for the *rpoB*516 and *katG*315 codons were initial denaturing at 96 °C for 3 min; 23 cycles of 95 °C for 50 s, 68 °C for 40 s, and 72 °C for 1 min; and final elongation at 72 °C for 7 min. For *rpoB*531 and 526 codons, the following cycling temperatures were used: initial denaturing at 95 °C for 5 min, 40 cycles of 94 °C for 1 min, 58 °C (for 531 codon) and 55 °C (for 526 codon) for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. Furthermore, initial denaturing at 95 °C for 5 min; 40 cycles of 95 °C for 30 s, 68 °C for 30 s, and 72 °C for 30 s; and final elongation at 72 °C for 7 min were used as cycling temperatures for amplifying the *rpoB*533 primer. All PCR fragments were analyzed by agarose gel electrophoresis using 1.5% agarose gel. The gels were exposed to UV light with photos taken by a GeneFlash gel documentation system (Syngene, USA).

## DNA sequencing

Two primers: *rpoB*-F (5´-TACGGTCGGCGAGCTGATCC-3´) and *rpoB*-R (5´-TACGGCGTTTCGATGAACC-3´) were used to amplify a 411 bp fragment (1119 – 1532 bp) of the *rpoB* gene, containing 81 bp (RRDR region). The region of the 435 bp (669–1103 bp) *katG* gene was amplified with a set of primers sequences: Forward-(5´-GCAGATGGGGCTGATCTACG-3´) and Reverse-(5´-AACGGGTCCGGGATGGTG-3´). Before sequencing, PCR amplification reactions were modified following (Farnia et al. 2010) and (Jaiswal et al. 2017). Final volumes of 50 µL were used for the *rpoB* and *katG* genes. For the *rpoB* gene, sterile DI water 32.9 µL, 10X buffer 5 µL, 5 U/µL Taq Polymerase 0.5 µL, 2.5 mM dNTPs 4 µL, 50 mM $MgCl_2$ 1 µL, 10 µM Forward and Reverse Primers 0.8 µL each and 4 µL of 20 ng DNA were used, while sterile DI water (25.75 µL), 10X buffer (6.25 µL), 5 U/ µL Taq Polymerase (0.5 µL), 2.5 mM dNTPs (4 µL), 50 mM $MgCl_2$ (2 µL), 10 µM forward and reverse primers (3.75µL for each) and 4 µL of 20 ng DNA were used to amplify the *katG* gene.

PCR conditions were as follows: initial denaturation at 95 °C for 5 min (*rpoB* gene) and 95 °C for 3 min (*katG* gene), followed by 40 cycles of 95 °C for 30 s, annealing at 69 °C for 30 s, extension of 72 °C for 30 s and final extension of 72 °C for 7 min for the *rpoB* gene followed by 35 cycles of 95 °C for 45 s, annealing at 64 °C for 45 s, extension of 72 °C for 1 min and final extension of 72 °C for 5 min for the *katG* gene. The PCR products were analyzed by electrophoresis on 2% agarose gel under ultraviolet light. After amplification, the PCR products containing specific genes and specific portions were sent to a sequencing company (Ward Medic), including the gel purification step.

## Sequence analysis

The sequencing data obtained from the company were aligned with the wild type H37Rv sequences of *rpoB* and *katG* genes (GenBank accession NC_000962.3) by using the BioEdit bioinformatic tool. Then, mutation codons and types of mutation in the samples were examined. For the *rpoB* gene, the nucleotide numbering system was based on *E. coli*.

Two isolates with novel mutation in the *katG*365 codon can be accessed (GenBank Accession No. MZ093466, MZ093467).

# Results

Out of 357 MTB isolates, 92 samples (25.8%) were found with drug resistance, as already described by (Rueangsak et al. 2020). Of the 92 drug-resistant samples, 38 (41.3%) were multi-drug resistant, while 46 (50%) and 8 (8.7%)

were mono-resistant to isoniazid and mono-resistant to rifampicin, respectively. Sixty-five drug-resistant samples (26 multi-drug resistant, 33 mono-resistant to isoniazid and 6 mono-resistant to rifampicin) were used to find mutations in the hot spot regions of the *rpoB* and *katG* genes.

### PCR amplification results

To detect mutations in the *rpoB*516, 526, 531, 533 and *katG*315 genes codons, each reaction for amplifying target size was optimized by using the H37Rv reference strain as the DNA template**.** Fifty-nine mono-resistant isoniazid isolates were detected by using the *katG*315 primer to determine the mutation codons. Among the 56 isoniazid resistant samples, 45 (80.4%) were identified as mutation in the *katG*315 codon. The remaining 3 samples were not differentiated by PCR.

Consequently, 32 mono-resistant rifampicin isolates were tested to observe mutations by using all *rpoB* primers individually. Of 32 RIF resistant samples, the *rpoB*516 gene mutation codon was found in 10 (31.2%), *rpoB*526 mutation codon was observed in 5 (15.6%) and 11 (34.4%) were identified as mutation in the *rpoB*531 gene codon. There were no mutation isolates with the *rpoB*533 primer. Therefore, the *rpoB*531 gene mutation codon had the highest percentage followed by *rpoB*516 and *rpoB*526.

Twenty-six MDR isolates were detected to observe combined mutation codons of both genes by using *rpoB*516, 526, 531, 533 and *katG*315 primers. A total of 13 out of 26 (50%) MDR cases showed mutations in both genes, while 4 isolates harbored no mutation in MDR samples. Mutation codon in the *katG* gene alone was found in 8 samples, whereas 1 sample was found to have mutation codon in the *rpoB* gene alone in MDR isolates. Six samples showed no mutation codon in mono resistant to INH and 3 samples of mono resistant to INH were not differentiated by PCR. Three samples were found as no mutation codon in mono resistant to RIF samples. Hence, 15 samples for the *rpoB* gene and 14 samples for the *katG* gene were selected for DNA sequencing to detect mutation sites that were not detected by PCR.

### DNA sequencing results

PCR amplifications for DNA sequencing of the *rpoB* and *katG* genes were optimized by using the H37Rv reference strain as the DNA template. Fifteen isolates that exhibited the *rpoB* gene with no mutation and 14 isolates that showed no mutation at *katG*315 codon by PCR were sequenced. Our analysis detected 5 mutated positions distributed among 4 codons within RRDR of the *rpoB* gene. Of the 15 isolates, 7 had mutation at codon 531 with amino acid changes at the position of TCG-TTG (Ser-Leu), while mutation at *rpoB*526 of CAC-CGC (His-Arg) occurred in 3 isolates and changes of CAC-TAC (His-Tyr) occurred in 2 isolates. Moreover, 1 mutation at *rpoB*516 GAC-GTC (Asp-Val) was identified, while a mutation at *rpoB*533 displayed CTG-CCG (Leu-Pro) amino acid change. No mutation was detected in 1 isolate.

Among the 14 isolates that showed no mutation *at katG*315 codon, 2 isolates had mutation at codon 365 CCG-CGG (Pro-Arg). These were recognized as novel mutations and no mutations were observed in 12 samples. The above 3 samples that were not differentiated by PCR revealed no mutation with the sequencing method.

### Distribution of mutation profiles based on PCR and DNA sequencing methods

All of mutation isolates detected by PCR and DNA sequencing were analyzed in percentage frequency showed by pie chart with individual gene codon (*katG* or *rpoB*) and multidrug resistant isolates. The *katG* gene mutations occurred in 47 (79.7%) of the 59 INH resistant samples. For the *rpoB* gene, 31 (96.9%) were observed as mutations in 32 RIF resistant isolates (Fig. 1). Finally, the number of those isolates were mapped to indicate the presence of geographical distribution of the *rpoB* and *katG* gene mutation samples in each province of Northeastern Thailand (Fig. 2).

## Discussion

This is the first study determining the distribution of *Mycobacterium tuberculosis* mutation genes codons in Northeastern Thailand. We found 41.3% as MDR, 50% as resistant to INH and 8.7% as resistant to RIF among 92 drug-resistant isolates. RIF resistance is caused by mutation in the *rpoB* gene, encoding the β-subunit of the DNA-dependent RNA polymerase (Jureen et al. 2006; Chaves et al. 2000). More than 95% of *rpoB* mutation codons in RIF-resistant clinical isolates have been found within the rifampicin resistant determining region (RRDR) (González et al. 1999). Our findings revealed that 96.9% of *rpoB* gene mutation codons were observed within the RRDR region. Our result was similar to a report in Bangladesh that also showed 96.43% of *rpoB* gene point mutation in the RRDR (Mistri et al. 2016). Other studies have documented the presence of common and novel *rpoB* mutations outside the RRDR (Mani et al. 2001; Aparna Lingala et al. 2010). According to our size of product, we only determined the most prevalent mutation codons between the positions 454–591 including the RRDR region. Inside the 81-bp RRDR, mutations at codons 516, 526 and 531 are responsible for up to 90% of RIF-resistant strains and recognized as the most frequent codons (Pang et al. 2013; Williams et al. 1994). Out of the *rpoB* codon mutation isolates in our study, 58.1% were detected at *rpoB*531 codon, 35.5% at *rpoB*516 codon and 32.3% at

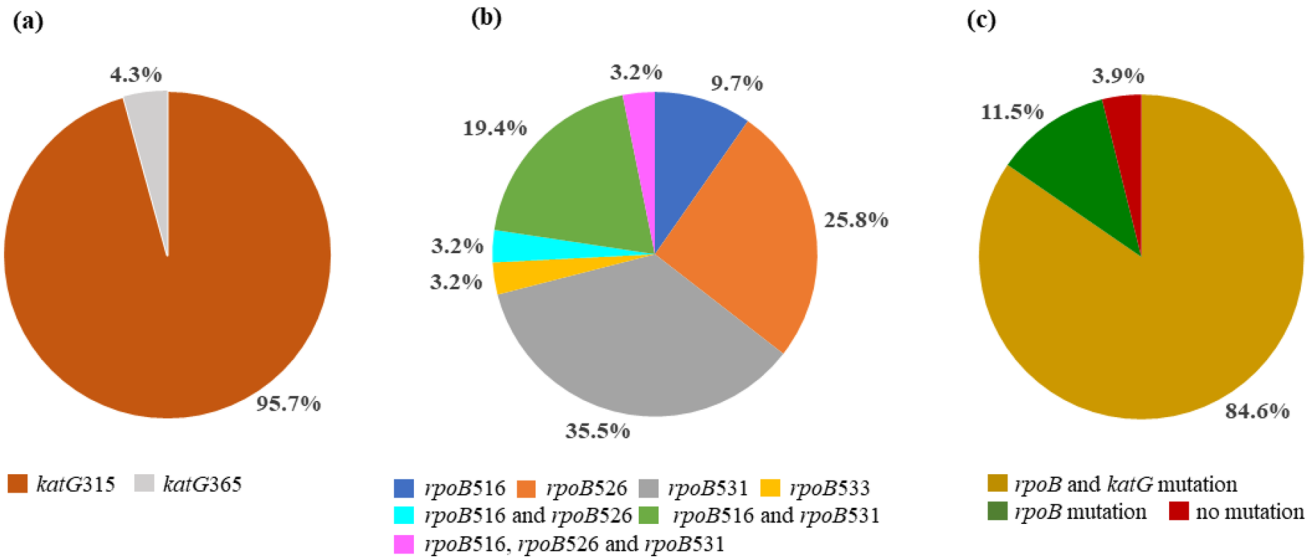


**Fig. 1** The mutation of assigned codon position in *katG* and *rpoB* genes were detected from clinical isolates of *Mycobacterium tuberculosis* by PCR and DNA sequencing methods. Mutation frequency percentage in the *katG* gene among 47 INH resistant isolates. Predominate mutation of *katG* gene found in codon position 315 (**a**). The *rpoB* gene mutation occurred among 31 RIF resistant isolates, including 23, 7 and 1 isolates were detected as single, double, and triple points mutation, respectively (**b**). Of 26 MDR isolates, 22 isolates were detected as *katG* and *rpoB* gene mutation by these 2 methods (**c**)

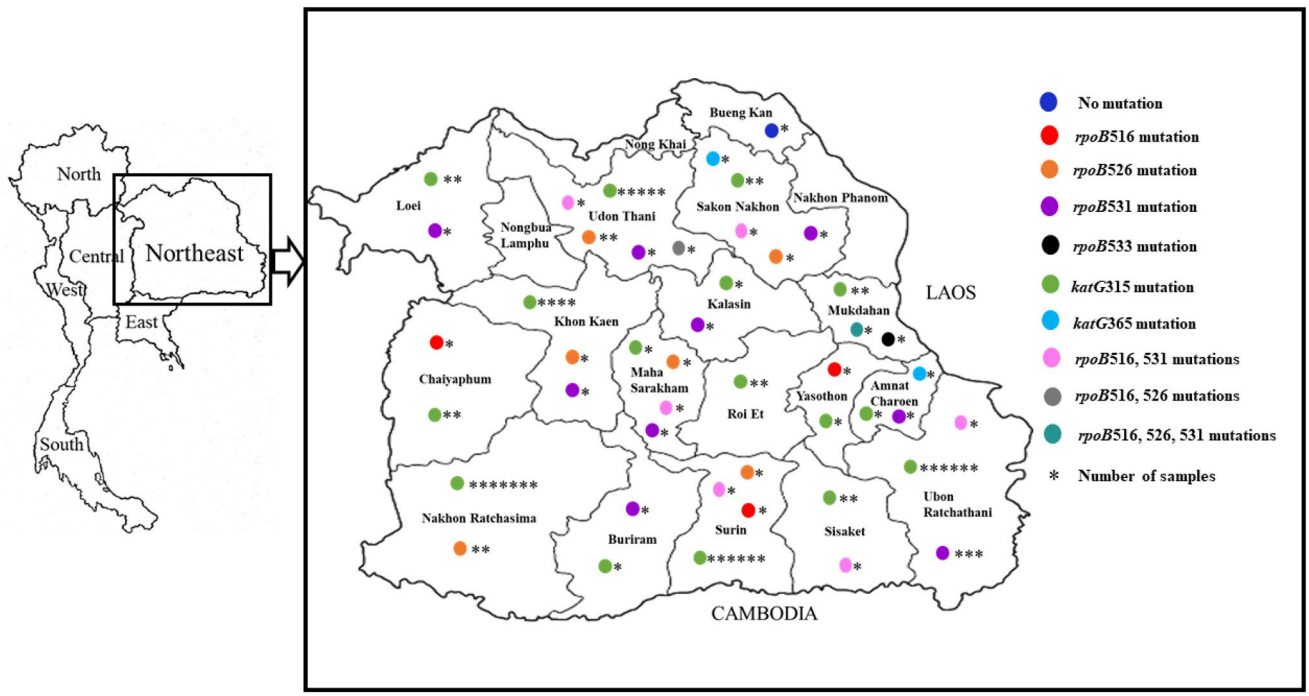


**Fig. 2** Geographical distribution of the *rpoB* and *katG* genes mutation samples found in each province of Northeastern Thailand region were indicated on map. The solid color circles were indicative of the presence of codon position of gene mutation. The asterisk indicates the number of mutation isolates

*rpoB*526 codon. Therefore, the most prevalent mutation codon was *rpoB*531, consistent with other findings in the Kyrgyz Republic (64.8%) (Isakova et al. 2018), Shanghai (53.8%) (Fan et al. 2003) and North India (66.7%) (Makadia et al. 2012). Most studies in China (Yue et al. 2003), Vietnam (Caws et al. 2006) and Singapore (Lee et al. 2005)

exhibited the *rpoB*526 codon as the second highest mutation frequency that is different from our results. Moreover, only 1 (3.2%) sample was found to have mutation in *rpoB*533 (Leu-Pro). This codon 533 was also detected in a study in Ratchaburi Province, Thailand as a novel mutation; however, different amino acids indicated *rpoB*533 (Leu-Arg) (Jintaridh et al. 2014). In accordance with our study, reports from Thailand by (Prammananan et al. 2008) and Taiwan by (Qian et al. 2002) displayed *rpoB*533 (Leu-Pro) mutation with the percentages of 2.1 and 3, respectively. Furthermore, double point mutation was determined in 7 samples and triple point mutation was identified in 1 sample in our study. In our research, *rpoB*531 codon that expressed highest mutation percentages, could be applied as an indicator to investigate rifampicin resistant *Mycobacterium tuberculosis* isolates in this area.

Nearly all rifampicin-resistant strains are further resistant to other drugs, especially to isoniazid. Thus, rifampicin resistance is recognized as a surrogate marker for MDR-TB (Traore et al. 2000). Detection of resistance for another drug in this study was isoniazid that inhibits the synthesis of mycolic acids and acts as a prodrug that is activated by the catalase-peroxidase enzyme, *katG* (Jena et al. 2014; Timmins and Deretic 2006). According to our report, the percentage of 79.7% for the *katG* gene mutation was consistent with earlier studies in Thailand (Boonaiam et al. 2010; Suthum et al. 2020) and other Southeast Asian Countries (Ismail et al. 2016; Caws et al. 2006; Valvatne et al. 2009; Liu et al. 2018; Cheng et al. 2021). The prevalence of *katG*315 mutation codon varied according to the geographical region: Southeast Asia (78.4%) (Seifert et al. 2015), Vietnam (85.3%) (Hang et al. 2019), China (59.4%) (Wu et al. 2006), the Netherlands (53%) (Van Soolingen et al. 2000), Japan (22%) (Ando et al. 2010) and Singapore (26%) (Lee et al. 1999). Furthermore, our results revealed that higher percentage of *katG*315 mutation codon in resistant to INH isolates than in MDR strains (53.3% and 46.7%, respectively). Two isolates of novel mutation at *katG*365 (GenBank accession no. MZ093466, MZ093467) with the amino acid substitution of CCG-CGG (Pro-Arg) were exhibited in our report. As previously mentioned, the *katG*315 mutation codon provided higher percentages, and therefore, this mutation codon could be utilized as an indicator for the detection of isoniazid resistance *Mycobacterium tuberculosis* isolates in this region.

After PCR amplification, 15 samples of resistant to RIF that did not show any mutation with primers of the PCR method were selected for *rpoB* gene sequencing to discover more mutation codons. These 15 isolates showed single point mutation within RRDR of the *rpoB* gene in 14 and no mutation in 1 sample. *katG* sequencing was performed in the 14 isolates (5 MDR samples, 6 samples were resistant to INH and 3 samples were not detected by PCR) that showed no mutation at *katG*315 codon. Mutation patterns performed by DNA sequencing in this study showed missense mutation in all isolates.

According to Fig. 2, mutation samples of the *rpoB* and *katG* genes in each province were widely distributed. The *katG*315 gene mutation samples were fully distributed throughout Northeastern Thailand and detected in every provinces except Bueng Kan that also showed no mutation samples. Moreover, *rpoB*531 gene mutation samples were also widespread and found in many provinces except for Bueng Kan, Chaiyaphum, Nakhon Ratchasima, Yasothon and Maha Sarakham. Higher distribution of mutation codons was found in provinces located near the Laos border. In Sakon Nakhon and Amnat Charoen Provinces, 2 isolates (one in each province) showing novel mutation were detected, while 1 isolate of *rpoB*533 gene mutation was found in Mukdahan Province.

When comparing PCR and DNA sequencing methods, discordant results were found for detection of the *rpoB* gene but not for the *katG* gene. PCR did not identify 7, 5, 1 and 1 isolates of *rpoB*531, *rpoB*526, *rpoB*516 and *rpoB*533 mutation codons, respectively. These observed possibly because the isolates contained heterogeneous populations of bacteria with mutated and wild type *rpoB* alleles, leading to amplification of the wild type PCR product (Ullah et al. 2019). No mutations were detected in 1 isolate of resistant to RIF and 12 isolates of resistant to INH by both PCR and DNA sequencing. This could be having mutations outside of the *rpoB* and *katG* genes that were not considered in this study. The DNA sequencing method showed greater sensitivity and specificity than PCR but the latter is more appropriate for use as a screening method to obtain faster results in low resource countries. Our results showed similar percentages of mutation codons reported in Thailand and Southeast Asian Countries except for *katG*315 mutation codon percentage and having novel mutation of *katG*365 codon.

Study limitations were as follows. Gene mutation of *katG*, *ahpC*, *inhA* and *kasA* can cause isoniazid resistance (Zhang et al. 1992); however, we only detected *katG* gene mutation that provided high-level resistance. Even though we identified the *katG* gene mutation, the amino acid change pattern of this gene mutation could not be performed by sequencing, especially in *katG*315 codon. Furthermore, a larger sample size would further validate our findings. We were unable to identify the entire genes of *rpoB* and *katG* because we focused on the most prevalent mutation sites for sequencing. Therefore, further studies should sequence the entire *rpoB* and *katG* genes to obtain more accurate results.

## Conclusions

Even though DNA sequencing has great accuracy, conventional PCR method could be applied as an initial marker for the screening of rifampicin and isoniazid resistant *Mycobacterium tuberculosis* in Northeastern Thailand. Our results showed that *rpoB*531 codon and *katG*315 codon expressed highest mutation percentages and could be utilized as indicators to investigate rifampicin and isoniazid resistant *Mycobacterium tuberculosis* isolates in this area. Two isolates with novel mutation at *katG*365, with amino acid substitution CCG-CGG (Pro-Arg) were exhibited in our report. The mutations reported here should be considered in the development of new molecular diagnostic methods for implementation in Northeastern Thailand. These novel techniques could reduce mortality and morbidity and provide effective treatment to ameliorate the formation of further resistant strains and spread of disease in Northeastern Thailand.

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**Data availability** Not applicable.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The author declares that they have no conflict of interest.

**Ethical approval** This study was approved by Khon Kaen University Ethics Committee for Human Research (HE631314).

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

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