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Genotypic Characterization of Multi-drug-Resistant *Mycobacterium tuberculosis* isolates in Myanmar

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Keywords: rifampicin, isoniazid, *Mycobacterium tuberculosis*, resistance, Myanmar

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Running Head: Multi-drug-resistant *M. tuberculosis* in Myanmar
Abstract

The number of multi-drug-resistant tuberculosis (MDR-TB) cases is rising worldwide. As a countermeasure against this situation, the implementation of rapid molecular tests to identify MDR-TB would be effective. To develop such tests, information on the frequency and distribution of mutations associating with phenotypic drug resistance in *Mycobacterium tuberculosis* is required in each country. During 2010, the common mutations in the *rpoB*, *katG* and *inhA* of 178 phenotypically MDR *M. tuberculosis* isolates collected by the National Tuberculosis Control Program (NTP) in Myanmar were investigated by DNA sequencing. Mutations affecting the 81-bp rifampicin (RIF) resistance-determining region (RRDR) of the *rpoB* were identified in 127 of 178 isolates (71.3%). Two of the most frequently affected codons were 531 and 526, with percentages of 48.3% and 14.0% respectively. For isoniazid (INH) resistance, 114 of 178 MDR-TB isolates (64.0%) had mutations in the *katG* in which a mutation-conferring amino acid substitution at codon 315 from Ser to Thr was the most common. Mutations in the *inhA* regulatory region were also detected in 20 (11.2%) isolates, with the majority at position -15. Distinct mutation rate and pattern from surrounding countries might suggest that MDR-TB has developed and spread domestically in Myanmar.
1. Introduction

In 2013, there were an estimated 9.0 million new cases and 1.5 million deaths from tuberculosis (TB), including TB-suffering HIV-positive people, globally [1]. TB is the second leading cause of death among infectious diseases worldwide. The increasing spread of multi-drug-resistant TB (MDR-TB), i.e. resistant to more than two drugs including isoniazid (INH) and rifampicin (RIF), along with the recent emergence of extensively drug-resistant TB (XDR-TB), which has exhibited an additional resistance to fluoroquinolone (FQ) and to at least one of the three injectable second-line drugs, possess a significant threat to tuberculosis control. The lack of adequate treatment, often due to irregular drug supply, inappropriate regimens or poor patient compliance, is associated with the emergence of problematic *M. tuberculosis* strains [1-2]. In 2010, there were an estimated 650,000 cases of MDR-TB among the world’s 12.0 million prevalent cases of TB [1].

MDR-TB cases, including those new and previously treated in 2002, were reported to be 4.2% and 18.4%, respectively, in Yangon, Myanmar [3-5]. The national drug resistant TB survey in 2002 also revealed those in the whole country to be 4.0% and 15.5%, respectively [6]. Hence, rapid identification of MDR-TB is crucial for proper treatment to avoid additional resistance development. In this context, the molecular characterization of drug resistance by identifying mutations in associated genes is applicable for developing potentially rapid molecular drug susceptibility tests as an alternative to conventional methods.

A convergence of data from different countries has indicated that resistance to RIF in 78% – 100% of cases is due to mutations resulting in an amino acid substitution within the 81-bp core region of the RNA polymerase β-subunit gene, or RIF resistance-determining region (RRDR) [7-21]. In contrast, INH resistance is mediated by mutations in several genes, most frequently within *katG*, which encodes a catalase-peroxidase that transforms INH into its active form, and in the regulatory region of *inhA*, which encodes a putative enzyme involved in mycolic acid biosynthesis. Mutations


in the \textit{inhA} regulatory region result in the overexpression of \textit{inhA} and INH resistance via a titration mechanism \cite{7, 14-24}.

The present study aims to determine the prevalence of resistance-associated mutations in three specific genes (\textit{rpoB}, \textit{katG} and the \textit{inhA} regulatory region) of MDR-TB isolates in Myanmar and compare the frequency of different mutations with those in isolates circulating in neighboring countries.

2. Materials and Methods

2.1. Isolates

A total of 178 MDR-TB clinical isolates, each corresponding to an individual TB patient, were randomly selected from the nation wide collection of the National TB Control Programme (NTP), Myanmar, during 2010. Drug susceptibility tests (DST) were carried out on Löwenstein-Jensen medium by the conventional proportional method at critical drug concentrations of 0.2, 2, 4, 40 µg/ml of INH, ethambutol (EMB), streptomycin (STR) and RIF, respectively \cite{9}. Laboratory capacity and quality assurance were controlled according to the supranational reference laboratory of tuberculosis in Bangkok and the Foundation for Innovative New Diagnostics (FIND).

2.2. DNA extraction

DNA was prepared for PCR using an EXTRAGEN MB DNA extraction kit (Tosoh Corporation, Tokyo, Japan), according to the manufacturer’s instruction.

2.3. Sequencing of \textit{rpoB}, \textit{katG} encoding regions and \textit{inhA} regulatory region

PCR was conducted with 20 µl of a mixture consisting of 0.25 mM of each dNTP, 0.5 M of betaine, 0.5 µM of each primer \cite{16}, 1 U of GoTaq DNA Polymerase (Promega, WI, USA), GoTaq buffer and 1 µl of DNA template. The reaction was carried out in a thermal cycler (Bio-Rad Laboratories, CA, USA) as follows: pre-denaturation at 96 °C for 60 s; 35 cycles of denaturation at 96 °C for 10 s; renaturation at 55 °C for 10 s; and elongation at 72 °C for 30 s, with a final extension at 72 °C for 5 min. The PCR products were separated by 1% agarose gel electrophoresis. DNA fragments of
interest were recovered from the agarose gel and used for sequencing according to the manufacturer's protocol with primers TB \textit{rpoB} S, TB \textit{katG} S and TB \textit{inhA} S for \textit{rpoB}, \textit{katG} and \textit{inhA}, respectively, a Big Dye Terminator v3.1 Cycle Sequencing Kit (Life Technologies Corp., CA, USA) and an ABI PRISM 3130xl Genetic Analyzer (Life Technologies Corp., CA, USA). Resulting sequences were compared with wild-type sequences of \textit{M. tuberculosis} H37Rv using BioEdit software (version 7.0.9) [25].

2.4. Comparison of the frequencies of the drug-resistance associating mutations

Fisher’s exact test was used to compare the drug-resistance associating mutations in this study with those in previous publications from surrounding countries. A two-tailed p-value less than 0.05 was considered statistically significant.

2.5. Ethical approval

Ethical approval is not required for the study as only clinical isolates of \textit{M. tuberculosis} already stored at National TB Control Programme were analyzed.

3. RESULTS

3.1. Drug susceptibility patterns

Among 178 MDR-TB clinical isolates, two isolates were resistant to only INH and RIF, 66 isolates showed additional STR resistance and the remaining 110 isolates were resistant to four first-line anti-TB drugs (Table 1).

3.2. Mutations in the \textit{rpoB} gene

Mutations in the RRDR of the \textit{rpoB} gene were identified in 127 isolates (Table 2). A single nucleotide alteration in codon 531, resulting in an amino acid substitution from Ser to Leu, was most prevalent and observed in 84 isolates (47.2%). The second most affected codon was 526, which was found in 25 isolates (14.0%) and showed six types of amino acid substitutions. Eight isolates had a mutation in codon 533, six had a mutation in codon 516, two had a mutation in codon 513 and one each in codons 510 and 517, respectively. Two of the isolates that had a mutation in codons 526, 516
and 533, also had an additional non-synonymous mutation in the RRDR (Table 2). No mutations were detected in 51 RIF-resistant isolates.

3.3. Mutations in the katG encoding region and inhA regulatory region

Out of 178 MDR isolates, 114 (64.0%) had an amino acid substitution in KatG, with the vast majority being Ser to Thr substitutions at the codon 315 (Table 3). Three isolates had a Ser to Asn substitution and a fourth had a Ser to Ile substitution at the same codon. Amino acid substitutions in KatG other than codon 315 were found in only one isolate: a Gly to Arg substitution at codon 285.

Mutations in the inhA regulatory region were observed in 20 (11.2%) MDR isolates, 18 of which had a C to T mutation at position -15, one had a G to T change at position -17 and one had a C to T mutation at position -15 in combination with a T to C mutation at position -8 (Table 3).

3.4. Frequencies of drug-resistance associating mutations

Frequencies of RIF- and INH-resistance associating mutations among the isolates in this study were compared with those among isolates from surrounding countries and Myanmar as presented in Table 4 and 5, respectively, and the results of Fisher’s exact test were shown in Table 6 and 7. The frequency of RIF-resistant isolates having mutations in RRDR was significantly lower than those in the studies from India [12], South China ([14]), Thailand ([11]), Bangladesh ([17]) and Myanmar (isolates from Yangon and Mandalay in 2013[26]) than those in our study. Mutation causing amino acid substitution at the position of codon 531 in rpoB is most frequently observed worldwide. The frequency of mutations at this position in this study was significantly lower than that in the studies from Myanmar in 2013 [26]. The frequency of INH-resistant isolates having mutations in katG at codon 315 or in inhA promoter region at the position of -15 was significantly lower than those in the studies from Thailand [22], Bangladesh [17] and Myanmar in 2013 [26]. In addition, the frequencies of mutations in katG at codon 315 was significantly lower than those in the studies from Bangladesh ([17]) and Myanmar in 2013 [26] than that in our study. In strong contrast, no significant difference was observed on both RIF- and INH resistance associating mutations between our study and the previous study from Myanmar on the isolates from Yangon in 2009.
Anti-tuberculosis drug resistance poses a significant threat to human health. It is usually developed due to the alteration of drug targets by mutations in *M. tuberculosis* chromosomal genes [7]. A large number of mutations in several genes that confer resistance to *M. tuberculosis* have been reported in different countries. One such study from populations in Myanmar [20] analyzed 29 MDR and 67 INH mono-resistant isolates. The study showed that 86.2% (25/29) of RIF resistant isolates had mutations in the RRDR of the *rpoB* gene, while 63.5% (61/96) and 2.1% (2/96) of INH resistant isolates had mutations at codon 315 of *katG* and a mutation in the regulatory region of *inhA*, respectively. Although the analysis seemed to be conclusive, the correlation rates between resistance and mutations in these genes were lower than those reported in countries Myanmar shares borders with: Thailand [11, 22], India [12, 23, 24], Southern China [14] and Bangladesh [17] (Tables 4 and 5). To strengthen the conclusions, the number of MDR isolates needed to be increased. Hence, we conducted molecular analysis of *rpoB* and *katG* encoding and *inhA* regulatory regions of 178 MDR isolates from Myanmar.

Consistent with previous studies that showed a large part of RIF-resistant *M. tuberculosis* isolates had mutations within the RRDR, in our study 71.3% of phenotypically RIF-resistant isolates carried mutations in the RRDR. This number is similar to that reported in the Philippines (69.7%) [8], Vietnam (74.3%) [15] and Korea (78.0%) [21], but lower than those reported in Thailand (96.1%) [11], India (96.7%) [12], Brazil (96.3%) [13], South China (95.0%) [14], Nepal (97.3%) [16], Bangladesh (92.7%) [17] where Thailand, India, South China and Bangladesh are sharing border with Myanmar. The present result was also lower than that of studies conducted in Yangon with samples collected in 2002 (86.2%) [20] and in Yangon and Mandalay in 2013 (100%) [26]. The difference in the sample collection period might have affected the mutation detection rate. Further, differences in the number of isolates analyzed in the previous study and ours (29, 33 and 178, respectively) might also be the reason of different mutation detection rate. We believe the
discrepancy between the DST and the sequencing results in 51 isolates could be due to the
coexistence of presence of wild type and resistant isolates in the initial culture [2], a mutation
outside of the core region of the rpoB gene [27, 28], the alteration of cell wall permeability or
metabolism of antibiotics [29, 30] or the problems in the quality control of drug susceptibility test.
Therefore, the presence of isolates lacking mutations should also be considered when using
commercially available gene diagnostic tools such as GenoType MTBDRplus (Hain Lifescience
GmbH, Nehren, Germany) and Xpert MTB/RIF (Cepheid, Sunnyvale, CA), or designing new
diagnostic tools for the detection of RIF-resistant TB.

The most frequently mutated codon in the RRDR in our study was codon 531 (48.3%),
which had a rate higher than those reported in clinical isolates from Poland (32.4 %) [10], North
India (38.7%) [18] and Vietnam (39.2%) [15], but lower than those reported in Brazil (56.1%) [13],
Bangladesh (57.4%) [17], Southern China (58.5%) [14], Thailand (58.5%) [11], Nepal (58.7%) [16],
India (59.0%) [12] and Morocco (59.6%) [9]. A much higher percentage of mutations at codon 531
was reported in Spain (72.3%) [19]. The second most frequently mutated codon was observed at
codon 526 (14.0%) in Myanmar, which also has been reported in many countries [11, 13-18]. The
sample collection site, Yangon, is a southern city in Myanmar and near to neighboring country
Thailand (Fig. 1). The distinct mutation pattern and mutation detection rate (Table 4) in isolates
from the surrounding countries might depict the low influence of neighboring countries.

Previous studies indicate that INH resistance is mediated by mutations in several genes, most
commonly katG, particularly at codon 315, and the regulatory region of inhA [7, 14-24]. Similarly,
we found that 64.0% and 11.2% of the phenotypically INH-resistant clinical isolates had point
mutations in katG and the inhA regulatory region, respectively. Furthermore, 49 INH-resistant
isolates had no resistant-associated alterations in the two targets. This indicates that the resistance in
these isolates could be due to mutations outside of the sequenced area or in other genes such as
oxyR-ahpC, kasA or ndh [7].
The amino acid substitution from Ser to Thr at residue 315 of KatG (KatG-Ser315Thr) has been predicted to be favored by *M. tuberculosis*, as this amino acid substitution appeared to reduce the activation of INH without abolishing the catalase-peroxidase activity of KatG, which has been recognized as a virulence factor [30]. However, the prevalence of KatG-Ser315Thr in clinical isolates around the world varies, especially with regard to the prevalence of TB. The present study documented the prevalence of KatG-Ser315Thr in 61.2% of INH-resistant isolates, which was quite similar to that of a previous study on the isolates collected from Yangon in 2002. The occurrence of KatG315 alteration was similar to those reported in India [12, 18] and South China [14] (Table 5), but not as high as those reported in isolates from Northeastern Russia (93.6%) and Yangon and Mandalay in Myanmar in 2013 (93.0 %) [26]. In general, a higher prevalence of this substitution has been observed in regions with a high incidence of TB compared with regions where the prevalence of TB is intermediate or low. And furthermore, this substitution was frequently seen in Beijing and MDR *M. tuberculosis* strains where strong correlation between Beijing genotype and MDR phenotype has been reported. [32]

Mutations in the regulatory region of *inhA* (preceding the mabA-*inhA* operon) that induce the overexpression of InhA have been known to contribute to INH resistance through an increased number of the target molecule [7]. Several studies from different countries have shown that about 10% – 34% of INH-resistant *M. tuberculosis* strains have mutations in the *inhA* regulatory region [7, 14-24]. We found a number within this range (11.2%). Four INH-resistant isolates carrying the KatG-Ser315Thr substitution also had a -15 C to T mutation in the *inhA* regulatory region. Additionally, one isolate possessed nucleotide substitutions -8 T to C and -15 C to T. Previous studies have shown that both inhA -8 T to C and -15 C to T are associated with a low-level resistance to INH [7]. It is possible that double mutations might cause an increased resistance to INH. Data showed the frequency of KatG-Ser315Thr and *inhA* -15 C to T mutations are different between Myanmar and its neighboring countries (Table 5). These data together with those of RIF resistance-associated mutations may depict a distinct emergence and evolution of MDR-TB in
Myanmar caused by recent political isolation. However, detailed comparison of genotyping data obtained by such as restriction fragment length polymorphism analysis or multiple-locus variable-number tandem repeat analysis is needed to conclude this.

**Conclusion**

This study provides valuable information on the mutations found in the *rpoB, katG* encoding and *inhA* regulatory regions in clinical isolates of *M. tuberculosis* in Myanmar. The results expand our current knowledge of the molecular mechanisms of drug resistance and also assist in improving current molecular-based techniques for the diagnosis of MDR tuberculosis fit to Myanmar. These improvements promise a more rapid detection compared with those achieved by methods based solely on the cultures of the isolates. Myanmar is a country bordered by China (northeast), India (northwest), Laos (mideast), Bangladesh (midwest) and Thailand (southeast) (Fig. 1). However, the mutation detection rate and the pattern of mutations are thought not to be affected by the surrounding countries. The political situation in Myanmar, which severely limits the movement of people, might be causing a distinct development and spread of multi-drug-resistant tuberculosis. To confirm this possibility, further information by molecular typing of MDR-TB strains circulating within Myanmar is necessary.

**ACKNOWLEDGEMENTS**

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for Zoonosis Control, Hokkaido University from MEXT, and in part by a grant from the U.S.-Japan Cooperative Medical Science Programs.

Conflict of interest statements: None of the authors have competing interests.
References


[10] Paluch-Oles J, Koziol-Montewka M, Magrys A. Mutations in the rpoB gene of rifampin-


\textbf{Figure legend}

Fig. 1. Myanmar and surrounding countries

Myanmar is a country bordered by China, India, Laos, Bangladesh and Thailand at northeast, northwest, mideast, midwest and southeast, respectively.
Table 1. Drug-resistance patterns of MDR *M. tuberculosis* isolates.

<table>
<thead>
<tr>
<th>Drug resistance pattern</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH + REF + STR + EMB</td>
<td>110</td>
</tr>
<tr>
<td>INH + REF + STR</td>
<td>66</td>
</tr>
<tr>
<td>INH + REF</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 2. Distribution of mutations in the rpoB RRDR of 178 MDR-isolates from Myanmar

<table>
<thead>
<tr>
<th>Affected codon(s)</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Number</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>From</td>
<td>To</td>
<td>From</td>
<td>To</td>
</tr>
<tr>
<td>510</td>
<td>CAG</td>
<td>CCG</td>
<td>Gln</td>
<td>Arg</td>
</tr>
<tr>
<td>513</td>
<td>CAA</td>
<td>CCA</td>
<td>Gln</td>
<td>Pro</td>
</tr>
<tr>
<td>516</td>
<td>GAC</td>
<td>GTC</td>
<td>Asp</td>
<td>Val</td>
</tr>
<tr>
<td></td>
<td>TAC</td>
<td>Tyr</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>517</td>
<td>CAG</td>
<td>CCG</td>
<td>Gln</td>
<td>Pro</td>
</tr>
<tr>
<td>526</td>
<td>CAC</td>
<td>TAC</td>
<td>His</td>
<td>Tyr</td>
</tr>
<tr>
<td></td>
<td>GAC</td>
<td>Asp</td>
<td>9</td>
<td>5.1</td>
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<td></td>
<td>CGC</td>
<td>Arg</td>
<td>5</td>
<td>2.8</td>
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<tr>
<td></td>
<td>CTC</td>
<td>Leu</td>
<td>4</td>
<td>2.2</td>
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<tr>
<td>531</td>
<td>TCG</td>
<td>TTG</td>
<td>Ser</td>
<td>Leu</td>
</tr>
<tr>
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<td>TGG</td>
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<td>0.6</td>
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<tr>
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<td>TTT</td>
<td>Phe</td>
<td>1</td>
<td>0.6</td>
</tr>
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<td>533</td>
<td>CTG</td>
<td>CCG</td>
<td>Leu</td>
<td>Pro</td>
</tr>
<tr>
<td>516/518</td>
<td>GAC/AAC</td>
<td>TAC/GAC</td>
<td>Asp/Asn</td>
<td>Tyr/Asp</td>
</tr>
<tr>
<td>516/533</td>
<td>GAC/CTG</td>
<td>GGC/CCG</td>
<td>Asp/Leu</td>
<td>Gly/Pro</td>
</tr>
<tr>
<td>526/527</td>
<td>CAC/AAG</td>
<td>CCC/CAG</td>
<td>His/Lys</td>
<td>Pro/Gln</td>
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<tr>
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<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Affected codon</td>
<td>Kat G Amino acid change</td>
<td>Nucleotide change</td>
<td>InhA regulatory region mutated position</td>
<td>Nucleotide change</td>
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<td>------------------------</td>
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<tr>
<td>285</td>
<td>Gly</td>
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<td>none</td>
<td>-15</td>
<td>C</td>
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<td>none</td>
<td>-15</td>
<td>C</td>
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<td>none</td>
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<td>-8/-15</td>
<td>T/C</td>
</tr>
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<td>none</td>
<td>none</td>
<td>none</td>
<td>-17</td>
<td>G</td>
</tr>
<tr>
<td>Mutated codon(s)</td>
<td>% Mutations in different geographic regions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>------------------------------------------</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>India (n=49 (121))</td>
<td>South China (n=60 (141))</td>
<td>Thailand (n=158 (11))</td>
<td>Bangladesh (n=218 (17))</td>
</tr>
<tr>
<td>530</td>
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<td>2.0</td>
<td>2.3</td>
<td>3.4</td>
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<tr>
<td>533</td>
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<td>22.9</td>
<td>22.9</td>
<td>17.2</td>
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<td>7.3</td>
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<td>531</td>
<td>3.5</td>
<td>10.0</td>
<td>2.0</td>
<td>7.3</td>
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</table>

* Including isolates having mutations at multiple codons.

* North India (n=110) and South India (n=39).

* Including isolates having no mutations.
<table>
<thead>
<tr>
<th>Locus</th>
<th>North India (n=121 [23])</th>
<th>South China (n=50 [14])</th>
<th>Thailand (n=160 [22])</th>
<th>Bangladesh (n=218 [17])</th>
<th>Myanmar (n=96 [20])</th>
<th>Myanmar (n=43 [31])</th>
<th>This study (n=178)</th>
</tr>
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<tr>
<td>kanG 315</td>
<td>55.4</td>
<td>60.0</td>
<td>79.4</td>
<td>83.9</td>
<td>63.5</td>
<td>93.0</td>
<td>63.5</td>
</tr>
<tr>
<td>inhA -15</td>
<td>24.7</td>
<td>8.0</td>
<td>10.6</td>
<td>16.5</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Others&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>36.0</td>
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<td>14.2</td>
<td>36.5</td>
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</tr>
</tbody>
</table>

<sup>a</sup> Including isolates having mutations at both loci.

<sup>b</sup> Including other mutations and no mutations.

<sup>c</sup> Not detected.
Table 6. Significance of the frequency of the mutations in Rif-resistant isolates in surrounding countries and other studies in Myanmar

<table>
<thead>
<tr>
<th>Mutated codons/absent a)</th>
<th>India b) (n=461 [145])</th>
<th>South China (n=60 [145])</th>
<th>Thailand (n=153 [145])</th>
<th>Bangladesh (n=218 [17])</th>
<th>Myanmar (n=50 [20])</th>
<th>Myanmar (n=33 [20])</th>
<th>This study (n=176)</th>
</tr>
</thead>
<tbody>
<tr>
<td>510</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>513</td>
<td>1.00</td>
<td>-</td>
<td>0.67</td>
<td>0.47</td>
<td>-</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>516</td>
<td>0.01</td>
<td>0.72</td>
<td>0.11</td>
<td>0.03</td>
<td>1.00</td>
<td>0.12</td>
<td>NA</td>
</tr>
<tr>
<td>517</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>526</td>
<td>0.06</td>
<td>0.83</td>
<td>0.01</td>
<td>0.03</td>
<td>0.58</td>
<td>0.00</td>
<td>NA</td>
</tr>
<tr>
<td>527</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>531</td>
<td>0.06</td>
<td>0.23</td>
<td>0.12</td>
<td>0.07</td>
<td>0.55</td>
<td>0.00</td>
<td>NA</td>
</tr>
<tr>
<td>533</td>
<td>1.00</td>
<td>1.00</td>
<td>0.23</td>
<td>0.42</td>
<td>1.00</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>other than QRDR b)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.50</td>
<td>-</td>
<td>NA</td>
</tr>
</tbody>
</table>

a) Corresponding L. coli numbering was used for rpoB.
b) North India (n=110) and South India (n=39).
c) Including isolates having no mutations.
Table 7. Significance of the frequency of the mutations in INH-resistant isolates in surrounding countries and other studies from Myanmar

<table>
<thead>
<tr>
<th>Locus</th>
<th>North India (n=121 [23])</th>
<th>South China (n=50 [14])</th>
<th>Thailand (n=150 [22])</th>
<th>Bangladesh (n=218 [17])</th>
<th>Myanmar (n=96 [20])</th>
<th>Myanmar (n=43 [26])</th>
<th>This study (n=178)</th>
</tr>
</thead>
<tbody>
<tr>
<td>katG 315</td>
<td>0.19</td>
<td>0.74</td>
<td>0.30</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
<td>NA</td>
</tr>
<tr>
<td>mfd-15</td>
<td>0.00</td>
<td>0.79</td>
<td>1.00</td>
<td>0.07</td>
<td>ND</td>
<td>0.77</td>
<td>NA</td>
</tr>
<tr>
<td>Others(^a)</td>
<td>0.90</td>
<td>0.38</td>
<td>0.00</td>
<td>0.00</td>
<td>0.2195</td>
<td>0.00</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^a\) Including isolates having mutations at both loci.

\(^b\) Including other mutations and no mutations.

\(^c\) Not detected.
Khin Saw Aye et al. Fig. 1