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CHARACTERIZATION OF MUTATIONS CONFERRING STREPTOMYCIN RESISTANCE IN *Mycobacterium tuberculosis* IN VIETNAM

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ABSTRACT

Recently, World Health Organization (WHO) has listed streptomycin (STR) in the list of second-line injectable drugs in the multidrug-resistant tuberculosis (MDR-TB) regimens and may replace amikacin under the same conditions. Nevertheless, molecular characterizations associated with STR resistance in the *Mycobacterium tuberculosis* population have not been fully investigated in Vietnam. The present study aimed to explore the variation and frequency of mutations in *rps*L and *rrs* genes and their relationship with drug-resistant patterns and *M. tuberculosis* genotypes in 163 STR-resistant strains from Vietnam. The mutation frequency of the *rps*L and *rrs* genes were 62% and 20.9%, respectively, and the mutation combination in both genes covered 81% of STR-resistant strains. The most prevalent mutations included *rps*L Lys43Arg (38.7%), Lys88Arg (19.6%), *rrs* A514C (10.4%) and A517C (5.5%). Thus, sequence analysis of *rps*L and *rrs* exhibited a sensitivity of 81% and specificity of 100% for the prediction of STR resistance in Vietnamese *M. tuberculosis* strains. The prevalence of STR-resistant mutations in double, triple and quadruple resistance strains was significantly different, compared with mono STR-resistant ones. Similarly, mutation frequency associated with STR resistance in MDR strains was significantly higher than that in non-MDR strains. In addition, the lineage 2 genotype was significantly correlated with a high rate of STR resistance-conferring mutation, as well as the mutation *rps*L Lys43Arg ($P < 0.01$), while the lineage 1 genotype was associated with a low rate of STR resistance-conferring mutation and *rrs* mutations ($P<0.05$). In conclusion, sequence analysis may be useful for the rapid detection of STR resistance in MDR *M. tuberculosis* strains, which in turn could contribute to better control strategies of TB in Vietnam. Other molecular mechanisms associated with STR resistance in STR-resistant strains without mutations in the *rps*L and *rrs* genes need to be further investigated.

Keywords: Streptomycin, multidrug-resistance, mutation, *rps*L, *rrs*, *Mycobacterium tuberculosis*, Beijing family, lineage 2 genotype.

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INTRODUCTION

Tuberculosis (TB) represents a major threat to human health in low and middle-income countries. World Health Organization (WHO) reported that one-third of the world's population is latently infected with *Mycobacterium tuberculosis*, the etiologic agent of the disease, and millions of lives are lost every year worldwide (WHO, 2022). Streptomycin (STR) was the first antibiotic used in the treatment of tuberculosis (TB) in the early 1940s (Mitchison, 1985). However, it was used as mono-therapy at that time which led to the rapid emergence of STR-resistant strains. The core of standard treatment regimens for treating adults with TB consists of an intensive phase of 2 months of isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB), followed by a continuation phase of 4 months of INH and RIF. Nevertheless, STR activity against *M. tuberculosis* has shown to be approximately equivalent to EMB, and therefore it has been used widely in many low and middle-income countries in the standard treatment regimen to replace EMB (Mitchison, 1985).

STR kills actively growing tubercle bacilli, but it is inactive against non-growing or intracellular bacilli (Mitchison, 1985). The drug interacts directly with the 30S subunit of the ribosome, thereby interfering with bacterial protein biosynthesis (Carter et al., 2000; Finken et al., 1993; Honore & Cole, 1994). The main targets of STR are 16S rRNA encoded by the *rrs* gene and ribosomal protein S12 encoded by the *rps*L gene (Carter et al., 2000). STR interferes with the protein synthesis by binding with the phosphate backbone of the 16S rRNA in the main domain encompassing the 530 and 915 loops of the *rrs* gene (Carter et al., 2000; Finken et al., 1993; Honore & Cole, 1994). This binding results in forming both salt bridges and hydrogen bonds, preventing contact with the S12 ribosomal protein and eventually leading to a misreading of the genetic code during translation (Carter et al., 2000). Mutations in *rps*L and *rrs* genes are major mechanisms of STR resistance (Fukuda et al., 1999; Sreevatsan et al., 1996). Mutations in the *rps*L gene are the most common, accounting for 19–78% of STR-resistant isolates, and are often associated with high levels of STR resistance. Mutations in the *rrs* gene are less frequent (10–28%), and often associated with low levels of STR resistance (Fukuda et al., 1999; Nhu et al., 2012; Sreevatsan et al., 1996). In addition, mutations in *gid*B, encoding for a 7-methylguanosine methyltransferase specific for the 16S rRNA, can confer a low level of STR resistance (Okamoto et al., 2007; Wong et al., 2011). Mutations in the *gid*B gene are often accompanied by mutations in the *rps*L and/or *rrs* gene. Since *gid*B mutations were also found in STR-sensitive isolates, their role in STR resistance need to be further investigated (Jnawali et al., 2013; Nhu et al., 2012; Wong et al., 2011). Taken together, approximately 48–93% of clinical STR-resistant isolates harbored STR-resistance mutations in *rps*L and/or *rrs* gene (Cuevas-Cordoba et al., 2013; Nhu et al., 2012; Sreevatsan et al., 1996). Moreover, the type and frequency of STR-resistance mutations vary according to geographical regions.

Globally, Vietnam ranks 10th among countries with the highest burdens of TB and one of 27 high MDR-TB burden countries. According to Viet Nam's Fourth National Anti-Tuberculosis Drug Resistance Survey conducted in 2011, the proportion of drug resistance among new and previously treated cases was 32.7% and 54.2%, respectively (Nhung et al., 2015). In addition, Vietnam is one of ten countries that make up 70% of the estimated new cases of multidrug-resistant (MDR) TB not enrolled in treatment, underlying that the transmission status of MDR-TB is mostly unknown. STR is still widely used in treatment regimens of drug-sensitive, drug-resistant and MDR TB cases. The prevalence of resistance to STR was 27.4% among new cases and 42.2% among previously treated cases (Nhung et al., 2015). Thus, STR can be used to treat at least 60% of STR-susceptible strains including drug-resistant and MDR forms. In 2019, WHO listed STR in the list of second-line injectable drugs

in the MDR-TB regimens and may replace amikacin under the same conditions when amikacin is not available or there is confirmed resistance to it (WHO, 2019). In order to introduce the drug properly and minimize the emergence of STR-resistant strains, molecular characterizations of STR resistance in *M. tuberculosis* strains need to be investigated. In this context, the present study aimed to explore the mutations in *rps*L and *rrs* genes of *M. tuberculosis* strains isolated in the period 2005–2009 to get insight into the genetic evolution associated with STR resistance before the limited use of this drug in the standard TB treatment regimen in Vietnam. In addition, the association of STR-resistance mutations with drug-resistant patterns and *M. tuberculosis* lineages are also investigated.

MATERIALS AND METHODS

Bacterial strains

A total of 260 clinical *M. tuberculosis* strains were randomly selected from the *M. tuberculosis* bank of the National Institute of Hygiene and Epidemiology (NIHE) in Ha Noi, Vietnam. These strains were collected from three National Tuberculosis Reference Laboratories including National Lung Hospital (North), Hue General Hospital (Centre) and Pham Ngoc Thach Hospital (South) by the Vietnam National Tuberculosis Control Program in between 2005–2009 and were transferred directly to NIHE. This sample set represented a mixture of all available drug-resistant profiles, including INH mono-resistant, RIF mono-resistant, non-MDR and MDR patterns according to phenotypic drug susceptibility testing. Then, all these strains were assigned into four groups according to STR susceptibility profiles including STR-sensitive, Mono-STR resistant, double-, triple- and quadruple-STR (resistant to one, two and three first line anti-TB drugs with additional resistant to STR). All the strains were sub-cultured on LJ medium. After 2–3 weeks of growth, the cultures were harvested and used for all the experimentations described below. All the bacterial cultures were performed in the Biosafety Laboratory level 3 of NIHE.

Ethics approval of research

This study has been performed in accordance with the Declaration of Helsinki. Since the study used only strains that were routinely collected from patients, informed consent to participate was not required. The Ethical Review Committee at the National Institute of Hygiene and Epidemiology (NIHE) approved the study procedures.

Drug susceptibility testing

Drug susceptibility testing (DST) was performed for the four first-line anti-TB drugs using the gold standard culture method as previously described (Nguyen et al., 2017). The critical concentrations of drugs as follow INH (0.2 mg/L), RIF (40 mg/L), STR (4 mg/L) and EMB (2 mg/L) were performed on LJ medium as recommended by the WHO. The H37Rv laboratory strain was included as a control for all the experiments.

DNA preparation and Molecular genotyping

M. tuberculosis colonies grown on LJ medium were harvested and suspended in 1 mL of TE buffer (10 mM Tris-HCl, 1 mM EDTA). After killing at 95 ^{o}C for 45 min (repeated twice), the suspension was centrifuged and the DNA-containing supernatant was transferred to a new tube and stored at -20 ^{o}C until use.

All the *M. tuberculosis* strains were classified and determined for the genotype by both a classical Spoligotyping technique combined with a Mycobacterial Interspersed Repetitive Units of Variable Number of Tandem Repeats (MIRU-VNTR) 24-locus method as described previously (Huy et al., 2017). The data were then compared with the online international databases on SITVIT WEB (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE) and MIRU-VNTR*plus* (http://www.miru-vntrplus.org/) for the *M. tuberculosis* lineage identification.

DNA sequencing and detection of STR-resistance mutations

The full length of the *rps*L gene was amplified and sequenced using specific primers Forward: 5'-GCGCCCAAGATAGA AAG-3' and reverse 5'-CAACTGCGATCCG TAGA-3'. In addition, a DNA fragment containing the loops 530 & 915 of the *rrs* gene was amplified and sequenced using primer pairs forward: 5'-GAGAGTTTGATC CTGGCTCAG-3' and reverse: 5'-CCAGGT AAGGTTCTTCGCGTTG-3' (Nguyen et al., 2017). The PCR reaction was prepared as follows: Each 25 µL of PCR mixture contained 2.5µL of 10X reaction buffer, 5 µL of 5X Q solution, 0.5 µL of 5 mM dNTPs, 0.5 µL of each forward and reverse primer (10 µM), 0.1 µL of 5 U/µL HotStar Taq (QIAGEN), 13 µL of H20 and 3 µL of DNA template. PCR conditions were 15 min of Taq activation at 95 ºC, and then 35 cycles of denaturation at 95 ºC, annealing at 58 ºC and extension at 72 ºC for 1 min/each step, followed by a final extension at 72 ºC for 5 min. PCR products were examined on 1.5% agarose gels before sending for purification and sequencing by Eurofins MWG Operon, Germany.

Each sequence was treated independently using the Bioedit software (version 7.1.10). The consensus sequence was generated for alignment and analysis. Point mutations were identified by comparison with the sequence of the *M. tuberculosis* H37Rv reference strain available in GenBank (NC.000962.3).

Statistical analysis

Sensitivity and specificity values were determined by comparison of phenotypic and genotypic data. The two-tailed Fisher's exact test was used to compare the mutation frequencies between drug-resistant patterns and between *M. tuberculosis* families. *P*-values < 0.05 were considered statistically significant. The odds ratio and 95% confidence interval (95% CI) were calculated to quantify the association of drug resistance patterns with mutation frequency.

RESULTS AND DISCUSSION

Prevalence of first-line drug resistance and *Mycobacterium tuberculosis* lineages

In this sample set, 205 *M. tuberculosis* strains were resistant to at least one of the four first-line anti-TB drugs and 55 strains were susceptible to all four drugs. The prevalence of STR resistance accounted for 62.7% (163/260) of the total population and 79.5% (163/205) of the drug-resistance strains. Interestingly, this *M. tuberculosis* population consisted of various resistance patterns to STR including mono-resistance (15.1%), double-resistance (9.3%), triple-resistance (10.7%) and quadruple-resistance (44.3%) (Table 1). Notably, 111 (68.1%) STR-resistant strains were MDR forms. Hang et al. 2013 reported that isoniazid and streptomycin resistance were observed in more than a quarter of newly diagnosed TB patients without treatment history in Hanoi, nevertheless, molecular characteristics associated with the STR resistance in this *M. tuberculosis* population have never been investigated (Hang et al., 2013). In other studies from the South of Vietnam, 116 out of 131 (88.5%) consecutive *M. tuberculosis* isolates resistant to either INH or RIF were resistant to STR (Nhu et al., 2012). According to the report of the fourth national anti-TB drug resistance survey in Vietnam, the proportion of STR resistance was 27.4% and 42.2% among new cases and previously treated cases, respectively (Nhung et al., 2015).

Pan-susceptible: sensitive to all four first-line drugs; Non-STR resistant: resistance to at least one of the four first-line drugs, except for STR; Double/Triple/Quadruple-resistant: combination patterns of STR resistance with one, two and three first-line drugs.

Analysis of spoligotyping and MIRU-VNTR data assigned *M. tuberculosis* strains into 3 major lineages among them, lineage 2 (Beijing family) was the most dominant (50%), followed by lineage 1 (EAI and EAI-like families, 29.2%), and lineages 4 (H, T, LAM and Unknown genotypes, 20.8%). The distribution of *M. tuberculosis* lineages

according to different drug-susceptibility patterns is shown in Table 1. Among drug-resistant strains, STR resistance was found in 36 (17.6%), 101 (49.2%) and 26 (12.7%) of lineage 1, lineage 2 and lineage 4 strains, respectively. The distribution of *M. tuberculosis* lineages is geographically different and lineage 2 is endemic in Vietnam. Previous studies have demonstrated that lineage 2 is strongly associated with STR resistance (Buu et al., 2012; Hang et al., 2013). Notably, lineage 1 and lineage 2 strains are the most dominant, covering up to greater than 80% of the total *M. tuberculosis* lineages circulated in Vietnam (Nguyen et al., 2016). Molecular epidemiology showed that lineage 2 is dominant in the North and South, while lineage 1 is prevalent in the Centre and South. Nevertheless, lineage 2 is rapidly spread through the country and is displacing the lineage 1 strains in Vietnam, particularly in the urban areas, and subsequently spread to rural areas where lineage 1 still dominates (Buu et al., 2009; Le Hang et al., 2021; Nguyen et al., 2012). Since the lineage 2 genotypes are often associated with young age, high virulence and multidrug resistance, this data suggests that molecular epidemiology studies of *M. tuberculosis* would be crucial for better control of the emergence of drug-resistant strains.

Table 1. STR-susceptibility patterns and its distributions among *Mycobacterium tuberculosis* genotypes

STR-susceptibility patterns	*Mycobacterium tuberculosis* genotypes			Total
	Lineage 1	Lineage 2	Lineage 4	
Pan-susceptible	20	14	21	55
Non-STR resistance	20	15	7	42
STR mono-resistance	11	14	6	31
Double-resistance	3	12	4	19
Triple-resistance	5	13	4	22
Quadruple-resistance	17	62	12	91
Total	76	130	54	260

STR-resistance mutations in the *rps*L gene

A total of 101 (62%) out of 163 STR-resistant strains revealed mutations in the *rps*L gene. The STR resistance-associated mutations in the *rps*L gene are described in Table 2. Since lineage 2 has a high prevalence in this sample set, there is a high prevalence of the *rps*L 43 mutations (39.3%), followed by mutations at codon *rps*L 88 (22.7%). The most common mutations were *rps*L Lys43Arg (38.7%) and Lys88Arg (19.6%), while mutations Lys43Asn, Lys88Met and Lys88Thr were less frequent, accounted for 0.6–2.6%. This result is totally concordant with previous studies in which the mutations *rps*L Lys43Arg and Lys88Arg have very low/no fitness cost compared to their STR-sensitive counterparts and above *rps*L mutant variants (Tsai et al., 2014). None of the drug-susceptible strains had any mutation in the *rps*L gene. A previous study from South Vietnam reported that the mutation frequency of codon 43 and 88 in the *rps*L gene of STR-resistant strains was 62.1% (72/116) and 18.9% (22/116), respectively, which is higher than that in our study (Nhu et al., 2012). Our finding is similar to studies from Thailand (63.6%), Myanmar (69.5%), is higher than studies from Mexico (19%) and Spain (24.6%), but it is lower than a study from China (79.4%) (Cuevas-Cordoba et al., 2013; Smittipat et al., 2016; Sun et al., 2016; Thida Oo et al., 2018; Tudo et al., 2010). Notably, the mutation *rps*L Lys43Arg was widely selected among STR-resistant strains in all *M. tuberculosis* populations from different geographic areas in the world.

Table 2. Mutations found in the *rps*L gene of STR-resistant strains and their distributions according to the *Mycobacterium tuberculosis* genotypes

Codon position(s)	Nucleotide change(s)	Amino acid change(s)	*Mycobacterium tuberculosis* genotypes, n (%)			Total n (%)
			Lineage 1 n = 36	Lineage 2 n = 101	Lineage 4 n = 26	
43	AAG-AGG	Lys-Arg	3	54	6	63 (38.7)
43	AAG-AAT	Lys-Asn	0	1	0	1 (0.6)
88	AAG-AGG	Lys-Arg	3	28	1	32 (19.6)
88	AAG-ATG	Lys-Met	1	2	1	4 (2.6)
88	AAG-ACG	Lys-Thr	1	0	0	1 (0.6)
Mutation frequency			8 (4.9)	85 (52.2)	8 (4.9)	101 (62)

STR-resistance mutations in the *rrs* gene

The frequency of *rrs* mutations was identified in 20.9% (34/163) STR-resistant strains. Mutations were mainly detected in two positions 514 (10.4%, 17/163) and 517 (5.5%, 9/163) of the *rrs* gene, while six other mutations displayed at positions 151, 239, 513, 878, 905 and 908 were also detected at low frequency (4.5%) (Table 3). Thus, mutations in loop 530 more commonly occurred than in loop 915 of the *rrs* gene. Only one STR-susceptible strain carried a new mutation at position 295C-T in the *rrs* gene. Since this mutation is located outside the STR resistance-conferring regions in the *rrs* gene, therefore it is probably not associated with STR resistance. Sun et al. (2016) reported only 7.2% (13/180) of STR-resistant strains from China carried mutations in the *rrs* gene, in which mutations were found in only three positions including 514, 517 and 906 (Sun et al., 2016). In Myanmar, the mutation frequency of the *rrs* gene in STR-resistant strains was relatively low (3.5%), and mutations were observed at positions 514, 517 and 905 (Thida Oo et al., 2018). A study from Singapore showed that 5/102 (4.9%) STR-resistant isolates carried either mutation 513 or 516 in the *rrs* gene (Sun et al., 2010). Overall, the mutation frequency and types of mutations in the *rrs* gene detected in our study were higher and more devise than previous reports from Singapore, China, Myanmar and Thailand, suggesting that lineage distributions of *M. tuberculosis* drive the selection of drug-resistance mutation patterns (Smittipat et al., 2016; Sun et al., 2016; Sun et al., 2010; Thida Oo et al., 2018).

Table 3. Mutations found in the *rrs* gene of STR-resistant strains and their distributions according to the *Mycobacterium tuberculosis* genotypes

Nucleotide position(s)	Nucleotide change(s)	*Mycobacterium tuberculosis* genotypes, n (%)			Total n (%)
		Lineage 1 n = 36	Lineage 2 n = 101	Lineage 4 n = 26	
151	C-G	0	1	0	1 (0.6)
239	C-T	1	0	0	1 (0.6)
513	C-T	0	0	1	1 (0.6)
514	A-C	4	11	2	17 (10.4)
517	C-T	6	1	2	9 (5.5)
878	G-A	2	0	0	2 (1.2)
905	C-G	1	0	0	1 (0.6)
908	A-C	1	1	0	2 (1.2)
Mutation frequency		15 (41.7)	14 (13.9)	5 (19.2)	34 (20.9)

Correlation between STR-resistance mutations, drug-resistance patterns and *Mycobacterium tuberculosis* genotypes

A total of 133 (81.6%) out of 163 STR-resistant strains revealed mutations in the *rps*L or *rrs* gene. Only two STR-resistant strains displayed mutations in both genes. The mutation frequency of the *rps*L gene in STR-resistant strains was significantly higher, compared with the *rrs* gene ($p < 0.01$). It is worth noting that mutations in the *rps*L gene are often associated with high level of STR resistance (Nhu et al., 2012; Sun et al., 2016). In addition, the mutation *rps*L Lys43Arg had no additional fitness cost in STR-resistant strains, leading to a broad transmission among clinical isolates (Spies et al., 2013). Mutations in the *rrs* gene are linked to a low level of STR resistance (Nhu et al., 2012; Sun et al., 2016), suggesting the mutations in this gene have a high biological cost and therefore are not favored in the selection of STR-resistant strains.

Table 4. Mutation frequency of *rps*L and *rrs* genes and their frequent mutations according to different STR-resistance patterns

Gene and frequent mutations	Frequency of STR-resistance mutations among drug resistance patterns (%)					
	Mono-R (n = 31)	Double-R (n = 19)	Triple-R (n = 22)	Quadruple-R (n = 91)	Non-MDR (n = 52)	MDR (n = 111)
*rps*L	41.9	63.2	54.5	70.3	51.9	66.7
Lys43Arg	25.8	42.1	31.8	44	32.7	41.4
Lys88Arg	12.9	21.1	22.7	20.9	17.3	20.7
rrs	19.4	15.8	22.7	19.8	15.4	22.5
A514C	6.5	5.3	9.0	12.1	5.8	12.6
A517C	9.7	5.3	4.5	4.4	5.8	4.5
*rps*L & *rrs*	58.1	78.9	77.3	90.1	67.3	87.4

Notes: Mono-R: resistant to only STR; double-R: resistant to STR combined with one first-line drug (isoniazid, rifampicin or ethambutol); Triple-R: resistant to STR along with a pair of first-line drugs; Quadruple-R: resistant to all drugs isoniazid, rifampicin, ethambutol and STR.

Mutation frequency in the *rps*L gene of double, triple and quadruple resistant strains was significantly higher than that in mono STR-resistant strains ($P < 0.01$) (Table 4). Similarly, the mutation frequency of codon *rps*L Lys43Arg was significantly higher in double, triple and quadruple resistant strains compared with mono STR-resistant strains ($P < 0.05$). Nevertheless, the difference in mutation frequency in the *rrs* gene was not significant among the STR-resistant patterns. Furthermore, the frequencies of mutations in both genes were significantly higher in MDR isolates than in non-MDR ones ($P < 0.01$), suggesting the effect of mutation accumulation can make the strains highly resistant to treatment (Nguyen et al., 2018).

For both genes *rps*L and *rrs*, the frequencies of mutations significantly vary according to geographic areas and *M. tuberculosis* genotypes (Cuevas-Cordoba et al., 2013; Lipin et al., 2007; Nhu et al., 2012; Springer et al., 2001; Sreevatsan et al., 1996). Global studies showed that in the regions where the lineage 2 strains (Beijing & Beijing-like families) are dominant, a high prevalence of STR resistance and mutations were observed. As the distribution of *M. tuberculosis* lineages are geographically different and lineage 2 is endemic in Vietnam, thus, *rps*L mutations were commonly detected compared with *rrs* mutations. In our samples, lineage 2 accounted for 50% of total STR-resistant strains which is totally in line with the link to mutation frequency in the *rps*L gene and its codon 43. Specifically, the mutation frequency in the *rps*L gene and codon 43 were significantly higher in lineage 2 than in all other lineages ($P < 0.01$). Conversely, the frequency of *rrs* mutations

was significantly higher in lineage 1 than in lineage 2 and 4 genotypes ($P < 0.03$). Overall, the frequency of STR-resistant mutations in both genes was significantly higher in lineage 2, compared with lineage 1 and 4 ($p < 0.02$). The finding in our study is consistent with the previous report that the propensity for acquiring drug-resistant mutations differs depending on *M. tuberculosis* lineages.

Altogether, the comparison of phenotypic and genotypic data exhibited sensitivity and specificity values of 81% and 100%, respectively, for the detection of STR-resistant *M. tuberculosis* in Vietnam. Thus, 19% of STR-resistant strains had no mutations in the *rps*L and *rrs* genes, possibly occurring in the *gid*B gene. The inability to sequence the *gid*B gene is a drawback of this present study. Nevertheless, global studies also reported at least 10−40% of STR-resistant isolates without any mutations in the *rps*L, *rrs* and *gid*B genes (Smittipat et al., 2016; Sun et al., 2016; Thida Oo et al., 2018), indicating that other potential STR-resistance mechanisms have not yet been discovered. Furthermore, the performance of *M. tuberculosis* susceptibility testing differs by platform and by the drug. The reliability of the phenotypic test as a gold standard may be questionable. Previous studies showed STR-phenotypic susceptibility testing was poor, particularly mostly using the solid proportion method (Banu et al., 2014). The present study highlights the potential use of molecular assay for rapid detection of STR resistance in MDR-TB patients, particularly in settings where STR susceptibility results are clinically important, which could contribute to better control of the spread of drug-resistant strains in Vietnam.

CONCLUSION

The present study showed that STR resistance in *M. tuberculosis* strains from Vietnam was strongly linked to mutations in *rps*L and *rrs* genes. In addition, the lineage 2 genotype was significantly related to STR resistance and *rps*L Lys43Arg mutation. Finally, mutation analysis of targeted genes could be feasible for the rapid determination of STR resistance in the MDR *M. tuberculosis* population of Vietnam.

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