EVALUATION OF MYCOBACTERIAL INTERSPERSED REPETITIVE UNIT-VARIABLE NUMBER TANDEM REPEAT TYPING TO DISCRIMINATE *MYCOBACTERIUM TUBERCULOSIS* STRAINS FROM MYANMAR

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Abstract. Mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing is a fast and promising method to discriminate *Mycobacterium tuberculosis* (MTB) strains. The present study was carried out to evaluate the applicability of MIRU-VNTR method for genotyping of clinical MTB strains from new pulmonary TB patients in Myanmar, and to compare the discriminatory ability between 15 loci and 24 loci MIRU-VNTR typing. One hundred and seven clinical MTB isolates which were collected during 2012-2013 were examined for anti-TB drug susceptibility using both proportion method and commercial line-probe assay. The results of internationally standardized 15 loci and 24 loci MIRU-VNTR typing methods were analyzed by a MIRU-VNTR *plus* web application together with Hunter-Gaston discriminatory index (HGDI) as numerical index to describe the discriminatory power. All tested MTB isolates showed unique patterns, which did not cluster and were distributed among 14 lineages. EAI genotype (30%) was the most prevalent genotype, followed by Beijing genotype (29%) that is significantly associated with multidrug-resistant TB (p <0.0004). Both 15 and 24 loci MIRU-VNTR were highly discriminatory (HGDI= 0.9833 and 0.9874, respectively). This study indicates that MIRU-VNTR could be used as a genotyping tool for Myanmar MTB strains.

Keywords: *Mycobacterium tuberculosis*, genotyping, MIRU-VNTR, Myanmar

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INTRODUCTION

Myanmar is one of the top 30 countries with a high tuberculosis (TB) and multidrug-resistant TB (MDR-TB) burden and the World Health Organization (WHO) estimated the incidence of TB in the country at 365/100,000 population in

the Global TB Report 2016 (WHO, 2016). Genotyping of *Mycobacterium tuberculosis* (MTB) plays an important role in TB control by identifying outbreaks, differentiating reactivation and/or reinfection and tracking transmission of drug resistant strains (CDC, 2004).

Mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing is a rapid and promising method to discriminate MTB strains and has been suggested as an acceptable alternative to the reference restriction fragment length polymorphism (RFLP) genotyping method (Supply *et al*, 2000, 2003; Dickman *et al*, 2010). Among more than 40 VNTR loci scattered on the MTB genome, 15 and 24 VNTR loci have been proposed as the international standards (Supply *et al*, 2006; Alonso-Rodriguez *et al*, 2008). However, their usefulness and discriminatory power should be evaluated in different settings and countries because diverse types of MTB strains have been reported from different geological areas and/or in different ethnic groups (Gagneux *et al*, 2006; Parwati *et al*, 2008).

Previous genotyping studies on Myanmar MTB strains reported that Beijing and East African Indian (EAI) strains are predominant genotypes (Phyu *et al*, 2003; Brudey *et al*, 2006; Phyu *et al*, 2009; Tun *et al*, 2017). Those studies focused mainly on MDR-TB isolates from previously treated patients and used spoligotyping as the main genotyping tool. However, spoligotyping does not have sufficient resolving power to discriminate Beijing family strains (Driscoll, 2009).

Hence, we evaluated the applicability of MIRU-VNTR method for genotyping of clinical MTB strains from new pulmonary TB patients in Myanmar, and compared the discriminatory ability between 15 loci and 24 loci MIRU-VNTR typing techniques. Establishment of MIRU-VNTR typing method with appropriate discriminatory power of prevailing MTB strains will encourage the use of this method for tracking recent transmission, finding TB outbreaks, distinguishing relapses or reinfections in Myanmar. It is also expected to fill the gap in the data of genotypes in newly diagnosed MTB strains in Myanmar.

MATERIALS AND METHODS

Specimen collection and culture

One hundred and seven MTB isolates from two sputum samples of newly diagnosed pulmonary TB patients attending tuberculosis centers in Yangon (Lathar Township) and Mandalay (Patheingyi Township), Myanmar during 2012-2013 were cultured in Lowenstein Jensen (LJ) medium (BD Difco, Sparks, MD) (GLI, 2014) at the National Tuberculosis Reference Laboratory (NTRL), Yangon or the Upper Myanmar Tuberculosis Laboratory (UMTL).

The study was approved by the Ethics Review Committee, Department of Medical Research, permit no. 54 Ethics 2012. Prior written consent was obtained from every participant.

Drug susceptibility test

Drug susceptibility testing was conducted with first line anti-tuberculosis drugs, namely, ethambutol (ETB), isoniazid (INH), rifampicin (RIF) and streptomycin (SM), using the standard agar proportion method (GLI, 2014). Resistant conferring mutations for INH and RIF were identified using Genotype MTBDR*plus* line-probe assay kit (Hain Lifescience, Nehren, Germany).

MIRU-VNTR typing

Mycobacterial genomic DNA was

extracted from bacterial colonies growing on LJ medium. Colonies were suspended in 300 µl aliquot of distilled water and incubated at 99°C for 20 minutes with mixing at 5 minutes intervals. DNA in supernatant was stored at -20°C until used. Mycobacteria strain typing by 15 loci MIRU-VNTR method employed 15 pairs of primers and additional 9 primer pairs for the 24 loci MIRU-VNTR typing (Supply, 2005). For each VNTR locus, 50 µl of PCR mixture containing HotstarTaq kit (Qiagen, Hilden, Germany) were subjected to the following thermocycling conditions (conducted in Veriti Thermal Cycler; Applied Biosystems, Foster City, CA): 95°C for 15 minutes; 40 cycles of 95°C for 1 minute, 59°C for 1 minute and 72°C for 1.5 minutes; with a final heating at 72°C for 10 minutes. Amplicons were separated by 3% agarose gel-electrophoresis at 50 volts for 2 hours, visualized by using Geldoc imager (Bio-Rad, Hercules, CA) and sizes were determined by comparing with 100 plus base pair DNA ladder (Bioneer, Daejeon, Korea). The sizes of repeat sequences were converted to allelic numbers according to Supply (2005) and the patterns analyzed using MIRU-VNTR*plus* web application (Weniger *et al*, 2010).

Similarity search was performed at distance <0.3, <0.4, <0.5 and <0.6 to compare with world's lineages. Dendrogram was generated using the UPGMA algorithm (Michener and Sokale, 1957). Discriminatory power analysis was conducted using Hunter-Gaston diversity index (HGDI) *D* as follows (Hunter and Gaston, 1988):

$$D = 1 - \frac{1}{N\,(N-1)} \sum_{j=1}^{S} n_j\,(n_j-1)$$

where N = total number of strains, S = total number of types described, and n_j = the number of strains belonging to the j^{th} type.

Allelic diversity of each MIRU-VNTR locus was classified as "highly discriminant" (HGDI D >0.6), "moderately discriminant" (0.3≤ HGDI D ≤0.6) and "poorly discriminant" (HGDI D <0.3) (Sola *et al*, 2003).

RESULTS

Genotypes

The best distance to obtain the optimal matching with global lineages was <0.6, which showed 83% similarity to 14 global lineages by the 24 loci MIRU-VNTR typing, with the most frequent being East African Indian (EAI) strain (30%), followed by Beijing strain (29%) (Table 1). There was no cluster and each strain had a unique MIRU-VNTR pattern based on phylogenetic analysis by UPGMA (data not shown).

Discriminatory power of MIRU-VNTR loci typing

Employing HDGI *D* values, apart from loci 577 (ETR C), 2687 (MIRU 24), 154 (MIRU 2), 3171 (Mtub 34) and 2059 (MIRU 20), which showed moderate discrimination, other loci exhibited high discriminatory power. Cumulative HGDI *D* value of the 15 and 24 loci MIRU-VNTR typing was 0.9833 and 0.9874, respectively (Table 2), with that for the Beijing and non-Beijing strains by 24 loci MIRU-VNTR of 0.9772 and 0.9877, respectively (Table 3).

Drug susceptibility

Of the 107 MTB isolates, 83 strains were pan-susceptible to the four first line anti-TB drugs (ETB, INH, RIF, and SM) tested, 24 strains resistant to one of the four drugs and 20 strains were MDR-TB (data not shown). The frequency of MDR-TB is statistically higher among Beijing

Table 1
Lineage similarity search by MIRU-VNTR*plus* at different matching distances between Myanmar *Mycobacterium tuberculosis* strains collected during 2012-2013 and global strains.

Lineages	Proportion of lineage matched at different distances (%)							
	<0.3		<0.4		<0.5		<0.6	
	24 loci	15 loci	24 loci	15 loci	24 loci	15 loci	24 loci	15 loci
Unknown	65	71	53	42	26	28	7	3
Multiple matches	-	-	2	2	3	8	9	23
Beijing	19	17	21	21	28	22	29	22
EAI	11	7	14	19	23	22	30	26
Delhi/CAS	1	1	2	2	5	3	6	5
LAM	-	-	1	2	3	3	4	3
NEW-1	1	1	1	1	2	1	2	1
URAL	-	-	-	1	1	2	2	2
Uganda-1	-	-	-	1	1	1	1	1
Uganda-2	-	-	-	1	-	1	-	1
Cameroon	-	-	-	3	1	3	1	4
Haarlem	-	1	1	1	1	1	1	1
S	-	-	1	1	1	1	2	1
X	-	1	-	1	1	1	1	2
TUR	-	-	-	-	1	-	1	-
H37Rv	3	1	4	1	4	3	4	1
West African 1	-	-	-	2	-	1	-	3
West African 2	-	-	-	-	-	-	1	-
Ghana	-	-	-	-	-	-	-	1
Canetti	-	-	-	-	-	-	-	1

MIRU-VNTR*plus* (http://www.miru-vntrplus.org/MIRU/index.faces).

than non-Beijing or unknown strains (chi-square statistic = 15.7229, *p*-value = 0.0004) (Table 4).

DISCUSSION

Among various genotyping methods, MIRU-VNTR typing has many advantages compare to RFLP typing or spoligotyping, such as requirement for low amounts of DNA, fast turn-around time, less labor demanding, and availability of a global database analysis network system allowing high discriminating power, particular among strains belonging to the Beijing lineage (Supply *et al*, 2001; Driscoll, 2009; Weniger *et al*, 2010). In Myanmar, there is limited information of the genetic diversity of MTB, especially by MIRU-VNTR typing method.

Using MIRU-VNTR*plus* web application, we compared similarities of local MTB strains with the global database based <0.6 matching distance with 24 loci and the 107 strains could be assigned to 14 lineages. Frequent movement of peoples from region to region and/or diverse ethnic groups in Myanmar might have resulted in the occurrence of various

Table 2
Discriminatory power of each locus and cumulative HGDI *D* of 15 and 24 loci MIRU-VNTR analysis of Myanmar *Mycobacterium tuberculosis* strains collected during 2012-2013.

15 loci MIRU-VNTR					
Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*
424 (Mtub04)	0.7748	1644 (MIRU 16)	0.6583	2996 (MIRU 26)	0.8589
577 (ETR C)	0.4132	1955 (Mtub21)	0.8339	3192 (MIRU 31)	0.721
580 (MIRU 4)	0.7448	2163b (Qub11b)	0.8648	3690 (Mtub39)	0.7404
802 (MIRU 40)	0.7306	2165 (ETR A)	0.7589	4052 (Qub26)	0.9007
960 (MIRU 10)	0.7708	2401 (Mtub30)	0.7235	4156 (Qub4156)	0.7098
				Cumulative HGDI *D* for 15 loci	0.9833
Additional 9 loci for 24 loci MIRU-VNTR					
Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*
154 (MIRU 2)	0.5198	2461 (ETR B)	0.7621	3007 (MIRU 27)	0.7145
2059 (MIRU 20)	0.4793	2531 (MIRU 23)	0.6364	3171 (Mtub34)	0.4484
2347 (Mtub29)	0.6785	2687 (MIRU 24)	0.514	4348 (MIRU 39)	0.7436
				Cumulative HGDI *D* for 24 loci	0.9874

genotypes.

All tested 107 strains revealed unique VNTR patterns that did not cluster. Zhang *et al* (2012) suggested that Beijing family strains are less likely to be clustered. The greater diversity observed in this strain might reflect the natural diversity in Myanmar, or the consequence of reactivation of latent MTB infections or increased global travels, which introduce new strains rather than transmission of the indigenous strains.

The overall discriminatory power of the 15 and 24 loci MIRU-VNTR typing methods as calculated using HGDI *D* value was considered as highly discriminatory. However, the HGDI *D* values of five loci, namely, VNTR 577 (ETR C), VNTR 2687 (MIRU 24), VNTR 154 (MIRU 2), VNTR 3171 (Mtub 34) and VNTR 2059 (MIRU 20), were in the less discriminatory range. In other studies in Southeast Asian countries, discriminatory power of these loci also are much lower (ranging from 0.00 to 0.26) (Kremer *et al*, 2005; Hyuen *et al*, 2013), which means that these loci might be less useful in discriminating strains from Asia where Beijing strains are predominant. This notion was supported by our study comparing the results of HGDI *D* value of Beijing and non-Beijing strains. This result suggests that another set of MIRU-VNTR loci should be applied to Myanmar TB strains.

EAI strain was the most prevalent in this study, followed by Beijing strain that also is present at high proportion in some Asian and Southeast Asian countries, such as China, and Thailand (van Soolingen *et al*, 1995; Lu *et al*, 2014; Srilohasin *et al*, 2014). Reports from 2003 and 2009 showed the EAI family is the most prevalent

Table 3
Discriminatory power of each locus and cumulative HGDI *D* of 15 and 24 loci MIRU-VNTR analysis of Myanmar *Mycobacterium tuberculosis* Beijing and Non-Beijing strains collected during 2012-2013.

Beijing strains 15 loci MIRU-VNTR					
Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*
424 (Mtub04)	0.604	1644 (MIRU 16)	0.3849	2996 (MIRU 26)	0.7355
577 (ETR C)	0.1269	1955 (Mtub-21)	0.6301	3192 (MIRU 31)	0.2925
580 (MIRU 4)	0.3226	2163b (QUB11b)	0.7247	3690 (Mtub39)	0.243
802 (MIRU 40)	0.3505	2165 (ETR A)	0.0645	4052 (QUB-26)	0.8581
960 (MIRU 10)	0.557	2401 (Mtub30)	0.1849	4156 (Qub4156)	0.3419
				Cumulative HGDI *D* for 15 loci	0.963
Additional 9 loci for 24 loci MIRU-VNTR					
Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*
154 (MIRU 2)	0.572	2461 (ETR B)	0.4237	3007 (MIRU 27)	0.6043
2059 (MIRU 20)	0.3613	2531 (MIRU 23)	0.4237	3171 (Mtub34)	0.3333
2347 (Mtub29)	0.471	2687 (MIRU 24)	0.1806	4348 (MIRU 39)	0.6839
				Cumulative HGDI *D* for 24 loci	0.9772
Non-Beijing strains 15 loci MIRU-VNTR					
Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*
424 (Mtub04)	0.663	1644 (MIRU 16)	0.6673	2996 (MIRU 26)	0.761
577 (ETR C)	0.4967	1955 (Mtub21)	0.8675	3192 (MIRU 31)	0.7701
580 (MIRU 4)	0.8106	2163b (Qub11b)	0.8693	3690 (Mtub39)	0.8022
802 (MIRU 40)	0.7689	2165 (ETR A)	0.8814	4052 (Qub26)	0.8681
960 (MIRU 10)	0.7284	2401 (Mtub30)	0.654	4156 (Qub4156)	0.6993
				Cumulative HGDI *D* for 15 loci	0.9839
Additional 9 loci for 24 loci MIRU-VNTR					
Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*	Locus (Allele)	HGDI *D*
154 (MIRU 2)	0.4471	2461 (ETR B)	0.8076	3007 (MIRU 27)	0.7326
2059 (MIRU 20)	0.5142	2531 (MIRU 23)	0.6189	3171 (Mtub34)	0.4549
2347 (Mtub29)	0.6443	2687 (MIRU 24)	0.5185	4348 (MIRU 39)	0.7483
				Cumulative HGDI *D* for 24 loci	0.9877

genotype in Myanmar, but more recent reports showed that the Beijing family is predominant in MDR-TB strains collected during 2012-2013 (Phyu *et al*, 2003, 2009; Tun *et al*, 2017). This study reveals Beijing and non-Beijing genotypes existed in almost equal proportion, suggesting that Myanmar MTB strains are changing over

Table 4
Drug susceptibility characteristics of Myanmar *Mycobacterium tuberculosis* Beijing and Non-Beijing strains collected during 2012-2013.

Genotype	MDR isolate number (%)	Non-MDR isolates number (%)		Total number (%)
		Resistant number (%)	Susceptible number (%)	
Beijing	13 (65)	0 (0)	18 (22)	31 (29)
Non-Beijing	6 (30)	3 (75)	49 (59)	58 (54)
Unknown + Multiple matches	1 (5)	1 (25)	16 (19)	18 (17)
Total	20 (100)	4 (100)	83 (100)	107 (100)

MDR, multidrug-resistant.

time. This might be due to i) the overall predominant genotypes are changing from EAI to Beijing, or ii) TB populations with different drug susceptibility characteristics (newly diagnosed TB *vs* MDR-TB) might represent different genotype dominance, Beijing strains being predominant in MDR-TB as reported from different geographical regions or TB management settings (Bifani *et al*, 1996; Agerton *et al*, 1999; Niemann *et al*, 2010). The present study showed that about two-third of MDR-TB from new TB cases belonged to the Beijing family and Beijing genotype is significantly associated with MDR-TB.

In conclusion, the present study lends support that the 15 and 24 loci MIRU-VNTR methods can be used to track recent transmission, finding TB outbreaks, distinguish relapses and reinfections in Myanmar with high confidence and discriminatory power (Supply *et al*, 2001). The study also reveals that it might be advisable to establish another set of MIRU-VNTR loci with more discriminatory power to distinguish and characterize Myanmar MTB isolates. However, the current findings should be tested on more samples to support the results and it will be necessary to establish a MTB strain bank system to collate past, current and future results, which will eventually contribute to appropriate TB management in Myanmar.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Agerton TB, Valway S, Blinkhorn RJ, *et al*. Spread of strain W, a highly drug resistant strain of *Mycobacterium tuberculosis*, across the United States. *Clin Infect Dis* 1999; 29: 85-92.

Alonso-Rodriguez N, Martinez-Lirola M, Herranz M, *et al*. Evaluation of the new advanced 15-loci MIRU-VNTR genotyping tool in *Mycobacterium tuberculosis* molecular epidemiology studies. *BMC Microbiol* 2008; 8: 34.

Bifani P, Plikaytis BB, Kapur V, *et al*. Origin and interstate spread of a New York City multidrug-resistant *Mycobacterium tuberculosis* clone family. *JAMA* 1996; 275: 452-7.

Brudey K, Driscoll JR, Rigouts L, *et al*. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol* 2006; 6: 23.

Centers for Disease Control and Prevention (CDC). Guide to the application of genotyping to tuberculosis prevention and control. Handbook for TB controllers, epidemiologists, laboratorians, and other program staff. Atlanta: CDC, 2004.

Dickman KR, Nabyonga L, Kateete DP, *et al*. Detection of multiple strains of *Mycobacterium tuberculosis* using MIRU-VNTR in patients with pulmonary tuberculosis in Kampala, Uganda. *BMC Infect Dis* 2010; 10: 349.

Driscoll JR. Spoligotyping for molecular epidemiology of the *Mycobacterium tuberculosis* complex. *Methods Mol Biol* 2009; 551: 117-28.

Gagneux S, DeRiemer K, Van T, *et al*. Variable host–pathogen compatibility in *Mycobacterium tuberculosis. Proc Natl Acad Sci USA* 2006; 103: 2869-73.

Global Laboratory Initiative (GLI). Mycobacteriolgy laboratory manual. Geneva: GLI, 2014. [Cited 2017 Mar 7]. Available from: http://www.stoptb.org/wg/gli/assets/documents/gli_mycobacteriology_lab_manual_web.pdf

Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol* 1988: 2465-6.

Huyen MNT, Kremer K, Lan NTN, *et al*. Clustering of Beijing genotype *Mycobacterium tuberculosis* isolates from the Mekong delta in Vietnam on the basis of variable number of tandem repeat versus restriction fragment length polymorphism typing. *BMC Infect Dis* 2013; 13: 63.

Kremer K, Au BK, Yip PC, *et al*. Use of variable-number tandem-repeat typing to differentiate *Mycobacterium tuberculosis* Beijing family isolates from Hong Kong and comparison with IS6110 restriction fragment length polymorphism typing and spoligotyping. *J Clin Microbiol* 2005; 43: 314-20.

Lu W, Lu B, Liu Q, *et al*. Genotypes of *Mycobacterium tuberculosis* isolates in rural China: using MIRU-VNTR and spoligotyping methods. *Scand J Infect Dis* 2014; 46: 98-106.

Michener CD, Sokal RR. A quantitative approach to a problem of classification. *Evolution* 1957; 11: 490-9.

Niemann S, Diel R, Khechinashvili G, Gegia M, Mdivani N, Tang YW. *Mycobacterium tuberculosis* Beijing lineage favors the spread of multidrug-resistant tuberculosis in the Republic of Georgia. *J Clin Microbiol* 2010; 48: 3544-50.

Parwati I, van Crevel R, Sudiro M, *et al*. *Mycobacterium tuberculosis* population structures differ significantly on two Indonesian islands. *J Clin Microbiol* 2008; 46: 3639-45.

Phyu S, Jureen R, Ti T, Dahle UR, Grewal HM. Heterogeneity of *Mycobacterium tuberculosis* isolates in Yangon, Myanmar. *J Clin Microbiol* 2003; 41: 4907-8.

Phyu S, Stavrum R, Lwin T, Svendsen OS, Ti T, Grewal HMS. Predominance of *Mycobacterium tuberculosis* EAI and Beijing lineages in Yangon, Myanmar. *J Clin Microbiol* 2009; 47: 335-44.

Sola C, Filliol I, Legrand E, *et al*. Genotyping of the *Mycobacterium tuberculosis* complex using MIRUs: association with VNTR and spoligotyping for molecular epidemiology and evolutionary genetics. *Infect Genet Evol* 2003; 3: 125-33.

Srilohasin P, Chaiprasert A, Tokunaga K, *et al*.

Genetic diversity and dynamic distribution of *Mycobacterium tuberculosis* isolates causing pulmonary and extrapulmonary tuberculosis in Thailand. *J Clin Microbiol* 2014; 52: 4267-74.

Supply P. Multilocus variable number tandem repeat genotyping of *Mycobacterium tuberculosis* technical guide. Lille: INSERM U629 Institut de Biologie/Institut Pasteur de Lille, 2005.

Supply P, Allix C, Lesjean S, *et al*. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2006; 44: 4498-510.

Supply P, Lesjean S, Savine E, Kremer K, van Soolingen D, Locht C. Automated high-throughput genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on mycobacterial interspersed repetitive units. *J Clin Microbiol* 2001; 39: 3563-71.

Supply P, Mazars E, Lesjean S, Vincent V, Gicquel B, Locht C. Variable human minisatellite-like regions in the *Mycobacterium tuberculosis* genome. *Mol Microbiol* 2000; 36: 762-71.

Supply P, Warren RM, Banuls AL, *et al*. Linkage disequilibrium between minisatellite loci supports clonal evolution of *Mycobacterium tuberculosis* in a high tuberculosis incidence area. *Mol Microbiol* 2003; 47: 529-38.

Tun T, Thinn KK, Aye KS, *et al*. Multi-drug resistant *Mycobacterium tuberculosis* strains in Myanmar patients. *Myanmar Health Sci Res J* 2017; 29: 552-7.

van Soolingen D, Qian L, de Haas PE, *et al*. Predominance of a single genotype of *Mycobacterium tuberculosis* in countries of east Asia. *J Clin Microbiol* 1995; 33: 3234-8.

Weniger T, Krawczyk J, Supply P, Niemann S, Harmsen D. MIRU-VNTR*plus*: a web tool for polyphasic genotyping of *Mycobacterium tuberculosis* complex bacteria. *Nucleic Acids Res* 2010; 1: 38.

World Health Organization (WHO). Global tuberculosis report 2016. Geneva: WHO, 2016. [Cited 2017 Mar 7]. Available from: http://www.who.int/tb/publications/global_report/en/

Zhang J, Mi L, Wang Y, Liu P, *et al*. Genotypes and drug susceptibility of *Mycobacterium tuberculosis* isolates in Shihezi, Xinjiang Province, China. *BMC Res Notes* 2012; 5: 309.