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The genotypic distribution of drug resistant Mycobacterium tuberculosis strains isolated from Northern region of Myanmar

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KEYWORDS
Mycobacterium tuberculosis; Drug resistant; Beijing; Lineages; HGDI.

ABSTRACT
Myanmar is one of both 30 high TB and MDR-TB burden countries worldwide. While most studies have expressed distribution of Mycobacterium tuberculosis genotypes in Lower Myanmar, there has been little research in genetic diversity of M. tuberculosis in Northern region. The objective of this study was to determine the genotypic distribution of drug resistant M. tuberculosis strains isolated from Northern region of Myanmar. Sixty-five isolates were randomly collected from TB Reference Laboratory of Northern region of Myanmar between August 2016 and December 2017. All isolates were genotyped by using 24-locus Mycobacterial Interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing. The results showed MIRU-VNTR typing classified 64 different patterns: 63 isolates had unique MIRU-VNTR profile and 2 isolates were grouped into one cluster. We found that the most prominent strains were Beijing lineages (n = 58, 89.23%) and the other included EAI (n = 2, 3.08%), Delhi/CAS (n = 1, 1.54%), and Unknown strains (n = 4, 6.15%). The overall discriminatory power of all strains showed 0.9995. The allelic diversity of each locus was predictable by HGDI index. Mtub21, Qub2163b, MIRU 26, QUB26 showed HGDI > 0.6 that were recognized as highly discriminatory power. In conclusion, 24-locus MIRU-VNTR offered high discriminatory power within tested isolates. Our findings showed Beijing genotypes were dominant in Northern region of Myanmar. The analysis of 24-locus MIRU-VNTR typing might be useful for broader understanding of TB outbreaks and epidemiology in this region.

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Introduction

Tuberculosis (TB) is a serious killer among the infectious diseases caused by *Mycobacterium tuberculosis* complex (MTB). Tuberculosis persists a major public health crisis all over the world[1]. Globally, 1.4 million people were predicted to lose their live with TB in 2015 and a 0.4 million of people living with HIV (PLHIV) died with TB. Additionally, the estimated 10.4 million was TB occurrence cases in 2015 worldwide[2]. In 2016, World Health Organization (WHO) reported that most anticipated TB incident cases were found within South East Asia region (45%), Africa (25%), Western Pacific (17%), Eastern Mediterranean (7%), Europe (3%), and the Americas (3%) respectively. Moreover, the top five countries including India, Indonesia, China, the Philippines and Pakistan which accounted for 56% of estimated cases[3]. Myanmar is one of both 30 high TB and MDR-TB burden countries[2]. It stood the fourth position with the higher prevalence rate, 525 cases per 100,000 populations compared with the global average of 178 cases per 100,000 populations in 2010. An anticipated 180,000 new cases occurred each year followed by 9,000 MDR-TB cases and 20,000 cases by TB co-infected HIV[4]. Myanmar is currently facing with the double burden of communicable diseases and non-communicable diseases. Diet style, less physical activity, tobacco usage and overdrinking alcohol are direct and indirect risk factors of TB and other health problems. Depletion of nutritional status such as protein energy malnutrition and micronutrient deficiencies are also the major causes of Tuberculosis in Myanmar. In recent years, Myanmar had increased the number of MTB strains which are resistant to drugs and co-infected with HIV[5].

The molecular epidemiology study of MTB is useful not only to examine the dispersion of tubercle bacilli in outbreaks but also to analyze the transmission of tuberculosis and to establish the risked aspects of tuberculosis among the community[6]. Spontaneously, there are several molecular typing methods now. The best approach to molecular method is whole genotype sequencing; however, WGS analysis is time consuming, costly and can examine only parts of the genome[7].

Other new developed molecular techniques for *M. tuberculosis* genetic classification are restriction fragment length polymorphism (RFLP)-IS*6110*, polymorphic GC-rich sequence (PGRS), pulsed field gel electrophoresis (PFGE), Spoligotyping (spacer oligo-nucleotide typing), ligation mediated (PCR), Mycobacterial interspersed repetitive unit (MIRU-VNTR) typing, amplification and sequencing of single nucleotide polymorphism (SNP) respectively[8]. MIRU-VNTR typing is currently used as standardized method and it is less laborious, has short time process, and the discriminatory power is similar in comparison with IS*6110*-RFLP typing, particularly if fully 24 loci are used[9]. The MIRU typing is based on variation in copy number of tandem repeat (VNTR) loci and it simply needs basic PCR and electrophoresis equipment. After introducing the standard sets of 12 loci and 15 loci MIRU-VNTR typing panels, 24 loci MIRU-VNTR typing is currently the best approach to discriminate strongly related strains[10]. Furthermore, it needs lower amount of DNA. Several reports were proved that 24-locus MIRU-VNTR typing is suitable for transmission of population-based studies[11].

In our study, MTB isolates were collected randomly from Northern region of Myanmar, TB reference Laboratory, Mandalay Region. These isolates were examined by 24-locus MIRU-VNTR typing to determine genetic diversity, to evaluate the discriminatory power of this method and to reveal transmission of some strains.

Materials and methods

Clinical isolates

A total of 65 drug resistant strain *M. tuberculosis* isolates were collected randomly from Northern region of Myanmar, TB reference laboratory between August 2016 and December 2017. All isolates were culture positive on Lowenstein Jensen medium and identified as MTB by using Capilia TB rapid test (MPB 64) and Niacin test (Biochemical test). Chromosomal DNA was extracted by using CTAB method for amplifying real time PCR[12]. This study protocol was approved by the Ethics Review Committee of the Department of Medical Research, Yangon, Myanmar (Ethics/DMR/2017/122) and the Khon

Kaen University Ethics Committee in Human Research, Khon Kaen, Thailand (Ethics number HE602220).

Drug susceptibility testing

Standard agar proportion method was used for susceptibility testing of first line anti-TB drugs performed on Lowenstein Jensen medium with the standard concentrations including ethambutol (ETB) 2.0 µg/ml, isoniazid (INH) 0.20 µg/ml, rifampicin (RIF) 40 µg/ml, and streptomycin (SM) 0.4 µg/ml[13]. Genotype MTBDRplus line-probe assay kit (Hain Lifescience, Nehren, Germany) was used for observation of resistant mutations for INH and RIF.

MIRU-VNTR genotyping

The 24-locus MIRU-VNTR typing was performed by PCR with specific primers including 24 loci that were described in Supply et al[14]. PCR premixes were prepared as following; one µl of DNA was added to a PCR master mix 49 µl (to the final volume of 50 µl). PCR master mixes 49 µl include 0.25 µl of Taq DNA polymerase (5 unit) (Invitrogen, USA), 8 µl of 1.25 mM dNTP (Sib enzyme), 5 µl of 10X PCR buffer, 5 µl of 10 µM each primer, and 1.5 µl of 50 mM $MgCl_2$. PCR was subjected to 40 cycles of conditions. The DNA of MTB genome H37RV was used as positive control and distilled water was used as negative control. PCR products were analyzed by electrophoresis on 1.5% agarose gels using 100 bp DNA ladder as standard size markers.

MIRU-VNTR analysis

Amplicon size was determined by Total Lab TL100 software, and obtained size was compared by applying online apparatus at (http://www.MIRU-VNTRplus.org) containing the allele for each locus by Supply et al. 2006. The number of alleles was filled into the indicated form according to the website instructor. The dendrogram was obtained using the UPGMA algorithm analysis.

Statistical analysis

The discriminatory power (the Hunter-Gaston discriminatory index [HGDI]) of each typing method was calculated according to a previously published method[15];

$$\text{HGDI} = 1 - \left[\frac{1}{N(N-1)} \sum_{j=1}^{s} xj(xj-1)\right]$$

Where D is the discriminatory power, N is the total number of isolates in the typing method, s is the number of distinct patterns discriminated by VNTR, and *j* is the number of isolates belonging to the *j*th pattern. The number of cluster strains in patients was used to calculate a rate of transmission, rather than progression to disease following infection in the past. Rate of transmission was calculated as follows[16]; (number of clustered strains-number of clusters)/ Total number of isolates x100. The percentage of clustering rate was calculated with following formula[17]; (c – c)/N x100, Where, N is the total number of cases in the sample, c is the number of clusters and *n*c is the total number of clustered cases.

Results

24-locus MIRU-VNTR

Among 65 isolates 24-locus MIRU-VNTR typing identified 64 different patterns. Four lineages were distributed containing Beijing (n = 58, 89.23%) and the other included EAI (n = 2, 3.08%), Delhi/CAS (n = 1, 1.54%), and Unknown strains (n = 4, 6.15%) (Table 1). Sixty-three isolates (96.92%) were unique (i.e., detected for only one strain) and only 2 isolates could be grouped into one cluster (Figure 1). The transmission rate was also evaluated by the formula as mentioned above in data analysis which showed 1.5% in this study.

Table 1 MIRU-VNTR fingerprinting patterns for 65 drug resistance *Mycobacterium* tuberculosis strains

[a]MIRU-VNTR pattern	Frequency	Lineages	[a]MIRU-VNTR pattern	Frequency	Lineages
2 4 4 2 4 2 3 5 2 5 3 4 2 2 5 1 5 3 3 5 3 7 2 3	1	[b]BJ	2 4 4 2 3 3 3 2 2 3 4 4 4 2 5 1 7 3 3 5 2 8 2 3	1	BJ
2 4 4 2 3 3 3 5 2 5 4 4 4 2 5 1 7 3 3 5 3 8 1 3	1	BJ	2 4 3 2 3 3 5 5 2 8 4 4 4 2 5 1 5 3 3 4 1 9 2 3	1	BJ
2 4 4 2 3 4 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 8 2 3	1	BJ	2 4 4 1 3 3 3 3 2 6 4 4 4 2 5 1 7 3 3 5 1 6 2 3	1	BJ
2 4 3 2 3 6 3 5 2 3 4 4 2 2 5 1 7 3 3 5 2 7 2 3	1	BJ	2 4 4 3 3 3 4 5 2 6 4 4 4 2 5 1 7 3 3 3 3 8 2 3	1	BJ
2 2 4 2 3 3 3 5 2 6 4 4 4 2 5 1 7 3 3 5 3 8 2 3	1	BJ	2 4 3 2 3 4 2 5 2 3 4 4 4 2 6 1 7 3 3 3 3 9 4 3	1	BJ
2 4 2 2 3 4 3 4 2 2 4 4 2 2 6 1 5 3 3 5 3 6 2 3	1	[c]Delhi/CAS	2 4 4 2 3 4 4 8 2 6 5 4 4 4 5 1 7 3 3 6 3 7 2 3	1	BJ
2 4 4 2 3 4 3 5 2 6 4 4 2 2 5 1 7 3 3 3 3 2 2 3	1	BJ	2 4 4 2 3 3 3 5 2 6 5 4 2 3 5 1 7 3 3 5 3 3 1 3	1	BJ
2 4 3 2 3 3 3 8 2 6 4 4 2 4 5 1 7 3 3 5 3 8 1 3	1	BJ	2 1 2 1 3 3 3 8 2 6 7 3 2 3 5 1 2 3 3 5 5 5 2 3	1	UK
2 4 4 2 3 3 3 5 2 6 5 4 4 4 5 1 7 3 3 5 3 3 4 3	1	BJ	2 4 4 1 3 3 3 3 2 6 5 4 3 2 5 1 5 3 3 5 3 7 2 3	1	BJ
2 4 4 2 3 2 3 3 2 6 3 4 2 2 5 1 7 3 3 5 3 8 1 3	1	BJ	2 4 4 2 3 2 4 5 2 6 4 4 3 2 5 1 5 3 3 5 3 7 1 3	1	BJ
2 2 4 2 3 3 2 2 2 6 5 4 2 2 5 1 5 3 3 3 3 5 2 3	1	[d]UK	2 4 4 3 2 3 3 5 2 6 8 4 2 3 5 1 2 3 3 5 3 6 2 3	1	UK
2 4 4 2 3 3 3 4 2 6 5 4 2 2 1 1 5 3 3 3 3 9 1 3	1	BJ	2 4 4 2 3 3 3 4 2 3 4 4 4 2 5 1 7 3 2 5 3 7 1 3	1	BJ
2 4 4 2 3 3 4 5 2 8 4 4 4 2 5 1 5 3 3 4 3 9 2 3	1	BJ	2 2 4 1 3 3 3 4 2 5 4 4 4 2 5 1 7 2 3 5 3 6 1 3	1	BJ
2 4 3 2 3 4 3 5 2 3 3 4 2 2 5 1 5 3 3 5 3 7 2 3	1	BJ	2 4 4 2 3 3 3 4 2 6 4 4 4 2 5 1 7 3 3 5 3 8 2 3	1	BJ
2 4 3 2 3 4 3 5 2 6 4 4 4 2 6 1 6 3 3 5 3 9 3 3	1	BJ	2 2 4 2 2 3 3 4 2 6 4 4 4 2 5 1 2 3 3 5 3 7 2 3	1	BJ
2 1 4 2 2 3 3 5 2 6 4 4 4 2 5 1 5 3 2 5 3 9 2 3	1	BJ	2 2 4 3 2 3 2 6 2 4 4 4 2 3 5 2 2 2 3 4 3 6 2 3	1	UK
2 4 6 2 3 3 3 4 2 6 4 4 4 2 5 1 7 3 2 5 3 7 1 3	1	BJ	2 4 4 2 3 3 2 5 2 4 4 4 4 2 5 1 5 3 3 5 3 8 2 3	1	BJ
2 4 4 2 3 3 3 5 2 8 4 4 4 2 5 1 5 3 3 5 2 7 3 3	1	BJ	2 4 4 2 3 4 2 5 2 6 4 4 4 2 5 1 7 3 3 5 3 8 2 3	1	BJ
2 2 4 3 3 4 3 7 2 3 6 3 4 2 6 1 2 3 3 3 4 6 1 3	1	[e]EAI	2 2 4 2 3 4 2 4 2 6 4 4 4 2 5 1 7 3 3 5 3 8 2 3	1	BJ
2 4 4 3 2 4 4 10 2 6 9 3 2 2 6 1 2 3 3 4 4 6 1 3	1	EAI	2 2 4 2 2 3 3 3 2 6 4 4 4 2 6 1 5 2 3 4 3 9 2 2	1	BJ
2 4 4 2 3 3 4 5 2 7 4 4 4 4 5 1 7 3 2 6 3 8 1 3	1	BJ	2 4 3 2 2 3 5 4 2 6 4 4 4 2 5 1 7 3 3 4 3 7 2 3	1	BJ
2 4 4 1 3 3 3 3 2 5 4 4 4 2 5 1 7 3 3 5 3 6 1 3	1	BJ	2 2 4 2 2 3 3 5 2 6 4 4 4 2 5 1 8 3 3 5 3 6 2 3	1	BJ
2 4 4 2 3 2 3 5 2 5 2 4 4 2 5 1 7 3 3 4 3 6 2 3	1	BJ	2 4 4 2 2 4 3 3 2 6 4 4 4 2 5 1 7 2 3 4 3 7 4 2	1	BJ
2 4 4 2 4 6 2 5 2 2 4 4 2 2 5 1 7 3 3 4 2 8 2 3	1	BJ	2 4 4 2 3 4 2 5 2 6 2 3 4 2 5 1 5 3 3 5 3 7 2 3	1	BJ
2 4 4 2 5 3 3 7 2 6 3 4 4 2 5 1 5 3 3 5 2 8 2 3	1	BJ	2 4 4 2 3 3 3 3 2 4 4 4 4 2 5 1 5 3 3 5 3 8 4 2	1	BJ
2 4 4 2 3 2 2 5 2 3 4 4 4 2 5 1 7 3 3 5 2 8 2 3	1	BJ	2 2 4 2 3 3 2 4 2 5 4 3 4 3 5 1 5 3 2 5 3 7 1 3	1	BJ
2 4 4 2 3 3 2 5 2 6 4 4 4 2 5 1 5 3 3 5 2 8 2 3	1	BJ	2 2 4 2 3 3 2 3 2 6 4 4 4 3 5 2 7 3 3 5 3 7 3 3	1	BJ
2 4 4 2 3 2 3 5 2 6 4 4 5 2 5 1 7 3 3 5 3 6 2 3	2	BJ	2 2 4 2 3 5 2 3 2 10 4 4 4 3 5 2 7 3 3 5 3 7 2 3	1	BJ
2 4 4 2 3 2 3 5 2 5 4 4 5 2 5 1 5 3 3 5 3 7 2 3	1	BJ	2 2 4 2 3 3 2 5 2 4 4 3 4 3 5 1 5 3 3 5 3 7 2 3	1	BJ
2 4 4 2 3 3 5 5 2 6 4 4 4 2 5 1 5 3 3 3 1 8 3 3	1	BJ	2 2 4 1 3 3 3 4 2 4 4 4 4 2 5 1 7 3 3 5 3 6 2 3	1	BJ
2 4 4 2 3 4 3 6 2 6 4 4 4 2 6 1 7 3 3 5 4 7 2 3	1	BJ	2 4 4 2 3 3 3 6 2 6 4 4 4 2 5 1 5 3 3 4 3 7 2 3	1	BJ
2 4 4 1 3 3 3 5 2 6 4 3 4 2 4 1 7 3 3 5 3 9 2 3	1	BJ			
2 2 4 2 3 3 3 2 2 3 4 4 4 2 5 1 7 3 3 5 2 9 2 3	1	BJ	**Total**	**64**	

Note: [a]MIRU pattern: MIRU 02, Mtub04, ETRC, MIRU 04, MIRU 40, MIRU 10, MIRU 16, Mtub21, MIRU 20, QUB2163b, ETRA, Mtub29, Mtub30, ETRB, MIRU 23, MIRU 24, QUB26, MIRU 27, Mtub34, MIRU 31, Mtub39, QUB26, QUB4156, MIRU 39. [b]Beijing strain. [c] Delhi-Central Asian strain. [d]Unknown strain. [e]East African Indian strain.

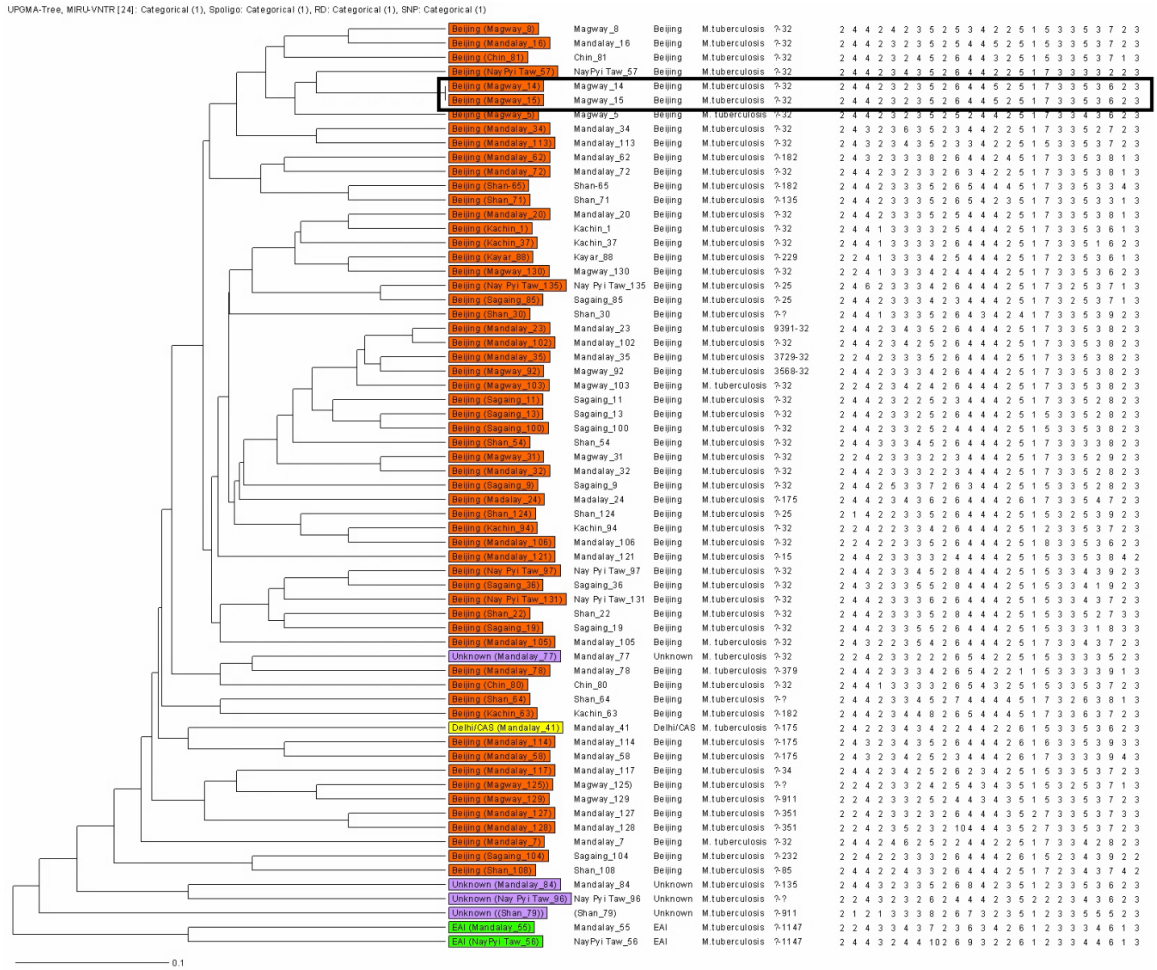

Figure 1 UPGMA tree show genetic relationship of all 65 drug resistant MTB strains including VNTR copy numbers and strain information. Large box indicated cluster isolates (MDR). Orange bars belong to Beijing lineages, Yellow (Delhi/CAS), Green (EAI), and Purple (Unknown).

Allelic profiles and discrimination

Allelic profiles and HGDI of 24-locus MIRU-VNTR for all MTB isolates in Northern region of Myanmar were summarized in Table 2. Allelic diversity was classified as highly discriminant (HGDI ≥ 0.6), moderately discriminant (0.3 < HDGI < 0.6), and poorly discriminant (HGDI ≤ 0.3)[18]. Mtub21, Qub2163b, MIRU 26, and QUB26 exceeded 0.6 that these were recognized as highly discriminatory power. Whereas 11 loci (Mtub04, MIRU 04, MIRU 40, MIRU 10, MIRU 16, ETRA, Mtub30, ETRB, MIRU 31, Mtub39, and Qub4156) were found moderately discriminative, the remaining 9 loci (MIRU 02, ETRC, MIRU 20, Mtub29, MIRU 23, MIRU 24, MIRU 27, Mtub34, MIRU 39) were found to be poorly discriminative. HGDI and cluster results based on the different set of MIRU-VNTR loci analysis of 65 MTB isolates from Northern region of Myanmar were shown in Table 3.

Table 2 Allelic diversity of 24 mycobacterial interspersed repetitive units (MIRUs) loci from 65 drug resistant tuberculosis strains

MIRU-VNTR locus	Allele number												Allelic diversity	Conclusion
	1	2	3	4	5	6	7	8	9	10	11	12		
MIRU 02		65											0.0	Poorly discriminant*
Mtub04	2	15	48										0.39	Moderately discriminant
ETRC		2	7	55		1							0.26	Poorly discriminant
MIRU 04	7	53	5										0.31	Moderately discriminant
MIRU 40		9	53	2	1								0.3	Moderately discriminant
MIRU 10		8	41	13	1	2							0.55	Moderately discriminant
MIRU 16		14	42	6	3								0.52	Moderately discriminant
Mtub21		3	9	11	33	3	2	3		1			0.68	Highly discriminant
MIRU 20		65											0.0	Poorly discriminant
QUB2163b		2	8	5	7	38	1	3		1			0.62	Highly discriminant
ETRA		2	4	49	6	1	1	1	1				0.41	Moderately discriminant
Mtub29			7	58									0.18	Poorly discriminant
Mtub30		15	2	45	3								0.46	Moderately discriminant
ETRB		53	8	4									0.31	Moderately discriminant
MIRU 23	1			1	56	7							0.23	Poorly discriminant
MIRU 24	62	3											0.07	Poorly discriminant
MIRU 26		6			22	1	35	1					0.58	Highly discriminant
MIRU 27		4	61										0.1	Poorly discriminant
Mtub34		5	60										0.13	Poorly discriminant
MIRU 31			7	10	46	2							0.45	Moderately discriminant
Mtub39	3	8	50	3	1								0.38	Moderately discriminant
QUB26		1	2		2	13	20	18	9				0.76	Highly discriminant
QUB4156	14	43	4	4									0.5	Moderately discriminant
MIRU 39		3	62										0.07	Poorly discriminant

Note: *Allelic diversity was classified as highly discriminant (HGDI ≥ 0.6), moderately discriminant (HGDI 0.3 < HDGI < 0.6), and poorly discriminant (HGDI ≤ 0.03).

Table 3 Hunter Gaston Discriminatory Index (HGDI) and cluster results based on different typing analysis of 65 drug resistant tuberculosis strains from Northern region of Myanmar

Typing methods	Total No. of patterns	No. of unique types	Total no. of clusters	Total no. of isolates in clusters (Cluster rate %)	Maximum no. of isolates in a cluster	HGDI
12 loci MIRU-VNTR	60 (N=65)	43	5	22 (33.8)	10	0.972
15 loci MIRU-VNTR	64 (N=65)	63	1	2 (1.5)	2	0.9995
24 loci MIRU-VNTR	64 (N=65)	63	1	2 (1.5)	2	0.9995

Drug resistance

Among all of 65 isolates, 31.4% (44/65 isolates) were accounted for resistant to both isoniazid and rifampicin and therefore, were known as MDR. 17 (26.15%) and 4 (6.15%) isolates were resisted to only isoniazid and rifampicin, respectively. Out of 44 MDR, 41 isolates were Beijing lineage, while only 3 were non-Beijing isolates (unknown strains). The MDR isolates were not found in EAI and Delhi/CAS lineage.

Discussion

24-locus MIRU-VNTR genotyping has developed a current standardized method and is presently useful for epidemiological study of *M. tuberculosis* thorough the world[19]. Total 65 isolates from Northern region of Myanmar between August 2016 and December 2017 were randomly collected. Sixty-one of 65 isolates (93.85%) could be classified into three lineages and the predominant genotype was Beijing (89.23%) which is highly prominent in Asian countries[20,21]. Remaining lineages distributed EAI (3.08%), Delhi/CAS (1.54%), and Unknown (6.15%). In contrast to a previous study, EAI lineage was the first predominance in Lower Myanmar, Yangon and the second was Beijing lineage[22]. The predominance of Beijing strains may be due to the human interaction with foreign countries such as China, India and Bangladesh.

The Beijing genotype is the most prominent genotype in Asia (Far East Asia, Middle East and Central Asia), Oceania and it is also emergent in other parts of the world[23]. The infection process of this lineage found to be related with immune response and it can control the macrophage-derived cytokines that was an important role in directing the immune response to a non-protective Th2 phenotype[24]. The relationship of Beijing genotype and drug resistance may have a certain tendency for acquiring drug resistance that were widely distributed (but not universal)[25]. In Myanmar, other previous studies described that the Beijing genotypes in MDR and XDR populations are highly prevalent[26,27]. Among Beijing isolates, 41 (70.69%) were infected with MDR, in which two strains were grouped into one cluster. Hence, Beijing strains in Northern region of Myanmar were highly correlated with Multi-drug resistance when compared with previous study in Yangon showed only 21.4%[22].

Allelic diversity of 24-locus MIRU-VNTR typing was analyzed by Hunter-Gatson Discriminatory Index. In the present study, alleles Mtub 21, Qub 2163b, MIRU 26 and Qub 26 found highly discriminatory power (HGDI ≥ 0.6). Some studies in China and India have expressed that those loci found highly discriminatory power[28,29]. A recent study in Myanmar has shown that Mtub21 found moderately discriminant (0.3 < HDGI < 0.6)[30]. On the other hand, the poorest discriminatory power was found in alleles MIRU 02, ETRC, MIRU 20, Mtub 29, MIRU 23, MIRU 24, MIRU 27, Mtub 34 and MIRU 39 (HGDI≤0.3). The remaining alleles Mtub 04, MIRU 04, MIRU 40, MIRU 10, MIRU 16, ETRA, Mtub 30, ETRB, MIRU 31, Mtub 39 and Qub

4156 showed moderately discriminant (HDGI ≥ 0.6). A previous study in Iran has found that MIRU 10 had high HDGI. However, it showed moderate discriminatory power in our study. HGDI of MIRU 20 and MIRU 02 were similar with the previous study of Iran[31]. The overall HGDI of total isolates in our study was 0.9995 that is similar to the previous studies[18,29].

In cluster analysis for all tested isolates, 64 different patterns were identified. One cluster of the obtained isolates shared MIRU patterns with two isolates, and 63 isolates (96.92 %) had unique patterns. Clustering rate of 65 isolates using 24 locus MIRU-VNTR typing showed only 3.1% belonged to Beijing strains. In previous study, the clustering rate of Beijing strains was nearly 80% compared with non-Beijing family that implies a high transmission rate[32]. Higher percentage of clustering rate indicates the possibility of related strains that are likely to spread other geographical areas. In our study, all resistant MTB isolates were genetically diverse and only one cluster was found. One cluster including two MDR strains (Beijing genotype) seemed to be acquired transmission of drug resistance strain. While tuberculosis is highly prevalent in Myanmar, the recent transmission rate in this study was 1.5%, which was lower than that described in some developed countries. For instance, one study in London stated that the recent transmission rate was 34%[33]. Another study in United States described that the proportion of TB cases attributable to recent transmission was 15%[34]. In China, the recent TB transmission rate was 13.34%[35]. The percentage of recent transmission was relatively low in our study, and this may indicate the higher possibility of recurrence in this study area.

Conclusion

This report describing the 24-locus MIRU-VNTR patterns and genetic diversity of *M. tuberculosis* genotypes from Northern region of Myanmar. Our findings revealed a diversity of strains and mode of transmission of some strains which can provide broader understanding of TB outbreaks and epidemiology. MIRU-VNTR typing is a useful tool to discriminate genetic diversity of MTB isolates in this area. Both 15 and 24-locus MIRU-VNTR typing show similar discriminatory power (HGDI 0.9995). The MIRU-VNTR locus that showed high discriminatory power (Mtub21, Qub2163b, MIRU 26, QUB 26 (h≥0.6)) can be applied as the first line locus for future studies. Beijing lineages isolates found to be MDR when compared with other lineage isolates. The lower clustering rate in our study indicates that acquired transmission occurred in this study period. Genotypic pattern proposes that the lower transmission rate may be due to higher possibility of reactivation cases in Northern region of Myanmar. Therefore, the information obtained from this study can be applied for future TB control studies.

Take home messages

The genotypic distribution of MDR-TB in Northern region of Myanmar was determined by 24-locus Mycobacterial Interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing. The most prominent strains were Beijing lineages and the other included EAI, Delhi/CAS, and Unknown strains. 24-locus MIRU-VNTR offered high discriminatory power (HGDI>0.6) within tested isolates.

Conflicts of interest

The authors declare no conflict of interest.

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