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Original Article

Genetic diversity of *Mycobacterium tuberculosis* using 24-locus MIRU-VNTR typing and Spoligotyping in Upper Myanmar

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Abstract

Introduction: MIRU-VNTR typing and Spoligotyping are the useful molecular tools for TB epidemiology study. Information regarding genetic diversity and tuberculosis (TB) transmission in Upper Myanmar only is scares.

Methodology: We determined the genetic diversity of *Mycobacterium tuberculosis* (Mtb) and TB transmission from Upper Myanmar TB Reference Laboratory, Mandalay Region, including Mandalay (72), Shan (22), Magway (15), Sagaing (13), Nay Pyi Taw (8), Kachin (7), Chin (2) and Kayah (1). One hundred and forty Mtb isolates were genotyped using 24-locus MIRU-VNTR typing and spoligotyping. Lineage classification and TB transmission analysis were performed.

Results: 24-locus MIRU-VNTR typing identified 135 unique profiles and two clusters compared to 35 spoligotyping profiles which contained 12 clusters and 23 unique isolates, Beijing (n=100, 71.4%) was found to be prominent lineage by combine two methods. The expected proportion attributable to recent transmission based on clustering rate was 2.1%. One cluster case was more likely to be in MDR patient.

Conclusions: Our findings showed Beijing genotypes were dominant in Upper Myanmar. The usage and analysis of 24-locus MIRU-VNTR typing might prove useful for our broader understanding of TB outbreaks and epidemiology than spoligotyping. The genotypic pattern of this combined method suggests that the lower transmission rate may be due to a higher possibility of reactivation cases in Upper Myanmar.

Key words: MIRU-VNTR; spoligotyping; Beijing; Lineages; HGDI.

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Introduction

Tuberculosis (TB) is a major public health problem worldwide, with an expected 10.0 million new TB cases and 1.3 million TB death cases as stated by the Global tuberculosis report 2018. Myanmar is one of the top 30 high TB and MDR-TB burden country with the estimated incidence TB rates at 358/ 100,000 populations and the incidence MDR/RR-TB rates at 26 cases per 100,000 populations in 2017 [1].

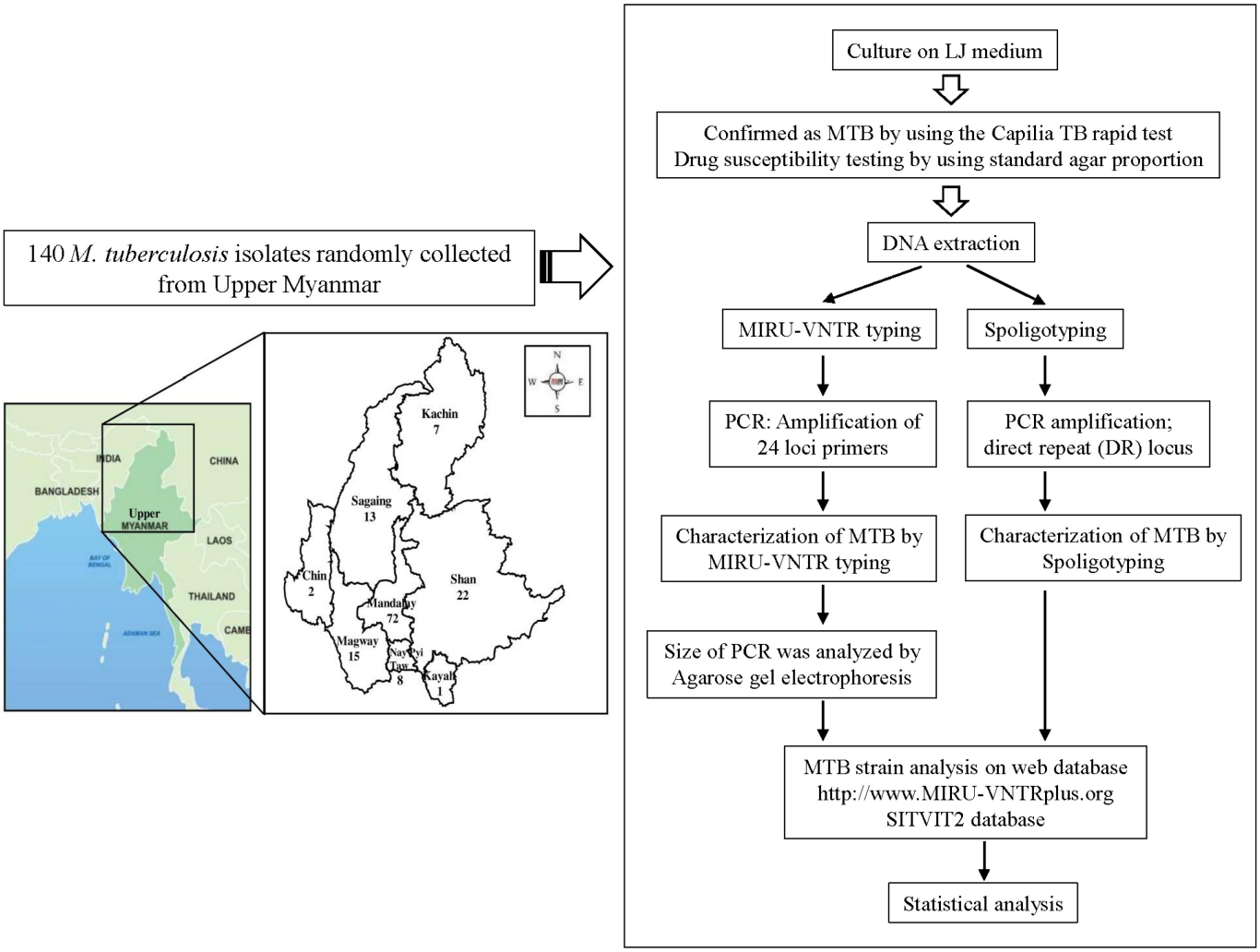
Molecular typing of MTB complex is a powerful tool to examine the dispersion of tubercle bacilli in outbreaks and to analyze the transmission of tuberculosis as well as to distinguish between recent TB infection and reactivation of a latent infection [2,3]. Several molecular methods for classification of MTB genotypes are restriction fragment length polymorphism (RFLP)-IS6110, polymorphic GC-rich sequence (PGRS), pulsed field gel electrophoresis (PFGE), spoligotyping, ligation mediated (PCR), Mycobacterial interspersed repetitive unit (MIRU-VNTR) typing, amplification, and sequencing of single nucleotide polymorphism (SNP) respectively [4]. IS6110-RFLP typing has been a gold standard method for genotyping, but the procedure is time consuming, labour intensive and technically demanding. It requires a huge amount of high quality DNA (2 µg) for enzyme digestion. The discriminatory power of this typing is not enough while strains with copy number IS6110 is 6 or less [5]. Furthermore, simple, quick and reasonable method based on PCR such as MIRU-VNTR typing and spoligotyping has been efficiently investigated the genetic relationship [6]. Spoligotyping is a simple, cost-effective PCR based method whose results are accurate and can be obtained within two days. It can detect the

presence or absence of 43 unique spacers with reverse line blot hybridization approach in the direct repeat (DR) locus of MTB complex [7]. The results are shown in the binary code that permits the re-construction of the large databases [8]. However, its discriminatory power is very low. The discriminatory power is increased when spoligotyping combined with MIRU-VNTR typing. Moreover, using the combination the two techniques of "MIRU-VNTR" typing and spoligotyping simultaneously could distribute the genomic outlines of MTB strains as compared to using only one method [9].

MIRU-VNTR typing is currently a standardized method and it is less laborious, much faster, and the discriminatory power is similar in comparison with IS6110-RFLP typing, particularly if 24 loci are analyzed [10]. MIRU typing relies on the variation in copy number of tandem repeat (VNTR) loci and it simply needs basic PCR and electrophoresis apparatus. After introducing optimized 12 loci and 15 loci MIRU-VNTR typing tools, 24 loci MIRU-VNTR typing has been found to currently be the best approach to discriminate strongly related strains [11]. Furthermore, it needs only a low amount of DNA. Several reports have shown that 24-locus MIRU-VNTR typing is suitable to examine transmission of strains in population-based studies [12].

Several genotyping studies have been carried in Yangon and the whole part of Myanmar, but data on the genetic diversity and transmission dynamics in Upper Myanmar only is rare. Previous studies of genotyping in Yangon, Myanmar reported that Beijing and East African Indian were the most predominant genotypes. Those studies observed that the combination of spoligotyping and MIRU-VNTR analysis may provide a better understanding of fingerprinting than using the combination of spoligotyping and IS6110-RFLP typing for epidemiological studies [13]. Till now there is no

Figure 1. Map of the Upper Myanmar showing the absolute number of MTB isolates per regions and states and research design.



published report for molecular typing of MTB isolates based on the combination of spoligotyping and MIRU-VNTR typing in Upper Myanmar. Hence, in this study we combined spoligotyping and MIRU-VNTR typing for determining genetic diversity of *M. tuberculosis* isolates in Upper Myanmar. This study is expected to help a better understanding in transmission mechanisms of MTB strains in this area.

Methodology

Clinical isolates and research design

A total of 140 *M. tuberculosis* isolates randomly collected from Upper Myanmar TB reference laboratory covering various regions of Upper Myanmar during January to December 2017 were recruited. The patients were from the following regions and states: Mandalay (72), Shan (22), Magway (15), Sagaing (13), Nay Pyi Taw (8), Kachin (7), Chin (2) and Kayah (1) (Figure 1). All isolates were culture positive on Lowenstein Jensen medium and identified as Mtb by using the Capilia TB rapid test (Neo, Shizuoka, Japan). Chromosomal DNA was extracted by using the CTAB method for amplifying real time PCR [14]. *M. tuberculosis* H37Rv was used as a reference strain. The research design of the study was shown in Figure 1.

Drug susceptibility testing

Standard agar proportion method was used for determination of susceptibility of first line anti-TB drugs and was performed on Lowenstein Jensen medium with the critical 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 [15]. Genotype MTBDRplus line-probe assay kit (Hain Lifescience, Nehren, Germany) was used for identification of resistant mutations for INH and RIF.

Spoligotyping

Spoligotyping was conducted as stated by the previously described standard protocol [6]. In brief, the direct repeat locus was amplified with primers Dra and Drb (biotinylated) using PCR and then the amplicons were hybridized to a set of 43 immobilized oligonucleotides covalently bound to the membrane. The hybridized PCR products were incubated with streptavidin peroxidase conjugate and the results were detected by chemiluminescence system (ECL detection). The spoligotype results in the binary format were compared with the SITVIT2 database and assigned the Spoligotype (or shared) International Types (SIT) code.

MIRU-VNTR typing

MIRU-VNTR typing was performed by PCR analysis with specific primers based on standard 24 loci defined in Supply et al [16]. 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 size markers. Amplicon size was determined by Total Lab TL100 software, and the obtained size was compared by applying online apparatus at (http://www.MIRU-VNTRplus.org). The dendrogram was obtained using the Un-weighted pair group method with arithmetic mean (UPGMA) algorithm analysis.

Data analysis

The discriminatory power (the Hunter-Gaston discriminatory index [HGDI]) of each typing method was calculated according to a previously published method [17];

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

Where HGDI 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 x_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 [18]; (number of clustered strains-number of clusters) / Total number of isolates ×100. The percentage of clustering rate was calculated with following formula [19]; (nc – c)/N ×100, Where, N is the total number of cases in the sample, c is the number of clusters and nc is the total number of clustered cases.

Statistical analysis

Demographic data and drug resistance MTB lineages were assessed by chi-square test using SPSS 20.0 software (SPSS Inc., USA). By p-value of less than 0.05 was considered statistically significant.

Results

Spoligotyping

Spoligotyping data of 140 isolates showed 35 spoligotype patterns: 19 (124 isolates, 88.6 %) patterns matched to SITs, while 16 (16 isolates, 11.4%) patterns were not matched any ST numbers and thus were known as "orphan" (Table 1). A further 117 (83.6%)

isolates were grouped into 12 clusters; 23 (16.4%) isolates were unique and an HGDI was 0.595.

The most predominant lineage was the Beijing family, accounting for 99 isolates (70.7%), including the major spoligotype patterns SIT1 (89 isolates) followed by EAI with 15/140 isolates (10.7%), CAS/Delhi with 6/140 isolates (4.3%) and T1 with 4/140 isolates (2.8%). The remaining 16 orphan isolates were unique types and the following SITs were more frequent such as SIT 292 (4 isolates), SIT 11, 89 and 1651 (each included 3 isolates) after SIT1. The clustering rate and transmission rate of spoligotyping was 88.6% and 80% respectively.

24-locus MIRU-VNTR typing

The analysis of MIRU-24 loci typing identified 137 different patterns while 135 isolates (96.4%) were unique (i.e., detected for only one strain) and only 5 isolates could be grouped into two clusters. One cluster contained three isolates and the other cluster contained two isolates. The clustering rate and transmission rate of 24-locus MIRU-VNTR was only 3.6% and 2.1%.

Allelic profiles and HGDI of 24-locus MIRU-VNTR for all MTB isolates in Upper Myanmar are summarized in Table 2. Allelic diversity was classified as highly discriminant (HGDI≥0.6), moderately discriminant (HGDI 0.3<HDGI<0.6), and poorly discriminant (HGDI≤0.03)[20]. The overall HGDI of all the loci sets reached 0.9996.

We paralleled the performance of the 24-locus MIRU-VNTR with the 15-locus and 12-locus sets combined with spoligotyping to determine the discriminatory power of different locus sets by MIRU-VNTR technique (Table 3).

Table 1. Spoligotyping profile of 140 isolates with ST number and orphan strains, which are not identified by a ST number. ST=shared type.

Spoligotypes	SITs	Spoligo Profile	Clades	No. of isolates
Clustered	1	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■■■	Beijing	89
	1651	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■□■■□■	Beijing	3
	190	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■□■■■	Beijing	2
	265	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■□■■■■■■	Beijing	2
	292	■■■■■■■■■■■■■■■■■■■■■■□■■■■■□□□□■□■■□■■■■■■	EAI	4
	11	■□□■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■□■■□□□■■■■	EAI	4
	89	■■□■■■■□□□□□□□□□□□□□□□□□□■■■□□□□■□■■■■■■■■■	EAI	3
	1409	■■■■■■■■■■■■■■■■■■■■■■□■■■■■□□□□■□■■□■□■■■■	EAI	2
	357	■■■□□□□■■■■■■■■■■■■■■■□□□□□□□□□□□□□□■■■■■■■	CAS	2
	1940	■□■□□□□■■■■■■■■■■■□□□□□□□□□□□□□□□□■■■■■■■■■	CAS	2
	53	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	T1	2
	167	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■■□□□□■■■■■■■	T1	2
Unique	255	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■□■■■■	Beijing	1
	269	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■	Beijing	1
	1364	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■□□■■■■	Beijing	1
	287	■■□■■■■■■■■■■■■■■■■□□■■■■■■■□□□□■□■■■■■■□■■	EAI	1
	1506	■■■■■■■■■■■■■□■■■■■■■■□■■■■■□□□□■□■■□■□■■■■	EAI	1
	26	■■■□□□□■■■■■■■■■■■■■■■□□□□□□□□□□□□■■■■■■■■■	CAS/Delhi	1
	428	■■■□□□□■■■■■■■■■■■■■■■□□□□□□□□□□□□■■□■■■■■■	CAS/Delhi	1
Unique	Orphan	■■■■■■■■■■■■■■■■■■■■■■□■■■■□□□□□□□■■□■□■■■■	Orphan	1
	Orphan	■□■■■■■■■■■■□■■■□■■■■■□■□□□□□□□□□□■■■■□■■■■	Orphan	1
	Orphan	■□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■	Orphan	1
	Orphan	■■■□□■□□□□□□■□□■■■■■■■■■■■■■□□□□■□■■■■□■■□■	Orphan	1
	Orphan	■■■■■■■■■□■■□□■■■■■■■■■■■■■■■■■■□□□□■■□■■■■	Orphan	1
	Orphan	■■■■■■■■■□■■■□■■■■■■■■■■■■■■■■■■□□□□■■□■■■■	Orphan	1
	Orphan	■■■■■■■■□□□■■■■■■■□■■■■■■□■■□□□□■□■■■■■□■■■	Orphan	1
	Orphan	■■■■■■■■■■■■■■■■■■■■■■■□■■■■■□□□□■□■■■■■■■■	Orphan	1
	Orphan	■■■■■■■■■■■■■■□■■■■■■■■■■■■■□□□□■□■■■■□■■■■	Orphan	1
	Orphan	■■■■■■■■■□■■■□□■■■■■■■■■■■■■■■■■□□□□■■□■■■■	Orphan	1
	Orphan	■■■■■■■■■□■■■□■■■■■■■■■□■■■■■□□□■□■■□■□■■□■	Orphan	1
	Orphan	■■■■■■■■■■■■■■■■■■■■■■□■■■■■□□□□■□■■□■□■■■■	Orphan	1
	Orphan	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■□■□□■	Orphan	1
	Orphan	□■■■■■■■■□■■■□□■■■■■□□□□■■■■■■■■□□□□■■□■■■■	Orphan	1
	Orphan	■■■■■■■■■□■■■□□■■■■■■■□■■■■■□□□□■□■■□■□■■□■	Orphan	1
	Orphan	■■□■■■■□□□□□□□□□□□□□□□□□□■■■□□□□■□■■■■□■■□■	Orphan	1

Table 2. Allelic diversity of 24 mycobacterial interspersed repetitive units (MIRUs) loci from 140 MTB isolates in Upper Myanmar.

MIRU-VNTR locus	Allele number												Allelic diversity	Conclusion*
	1	2	3	4	5	6	7	8	9	10	11	12		
MIRU 02		140											0.0	Poorly
Mtub 04	10	39		91									0.49	Moderately
ETRC		6	10	122	1	1							0.23	Poorly
MIRU 04	14	102	9	7	7	1							0.45	Moderately
MIRU 40		21	112	3	4								0.33	Moderately
MIRU 10		17	86	28	6	3							0.56	Moderately
MIRU 16		32	98	6	3	1							0.45	Moderately
Mtub 21		5	10	21	78	10	4	6	2	3		1	0.65	Highly
MIRU 20		140											0.0	Poorly
Qub 216b		9	16	9	12	81	3	4	3	2	1		0.63	Highly
ETR A		3	7	101	10	7	2	4	6				0.46	Moderately
Mtub 29		18	122										0.22	Poorly
Mtub 30	2	45	2	88	3								0.5	Moderately
ETR B		112	13	5	7	3							0.34	Moderately
MIRU 23	1			1	120	17	1						0.25	Poorly
MIRU 24	136	4											0.05	Poorly
MIRU 26	1	25	3		45	2	59	4	1				0.68	Highly
MIRU 27	1	21	118										0.26	Poorly
Mtub 34		10	130										0.13	Poorly
MIRU 31		2	23	25	85	5							0.57	Moderately
Mtub 39	3	16	105	9	2	4	1						0.41	Moderately
Qub 26		3	6	2	4	33	36	43	13				0.77	Highly
Qub 4156	36	91	8	5									0.5	Moderately
MIRU 39		11	129										0.14	Poorly

* 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 140 MTB isolates from Upper 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
Spoligotyping	35 (N = 140)	23	12	124 (88.6)	89	0.595
12 loci MIRU-VNTR	105 (N = 140)	92	13	48 (34.3)	10	0.9898
Spoligotyping +12 loci MIRU-VNTR	111(N = 140)	102	9	38 (27.1)	10	0.991
15 loci MIRU-VNTR	137 (N = 140)	135	2	5 (3.6)	3	0.9996
Spoligotyping +15 loci MIRU-VNTR	137 (N = 140)	135	2	5 (3.6)	3	0.9996
24 loci MIRU-VNTR	137 (N = 140)	135	2	5 (3.6)	3	0.9996
Spoligotyping +24 loci MIRU-VNTR	137 (N = 140)	135	2	5 (3.6)	3	0.9996

Table 4. Drug resistant patterns between lineages and clusters based on combined typing.

Drug resistance patterns	Beijing (N = 100)	Non- Beijing (N = 40)	p-value	Clusters (N = 5)	Non-Clusters (N = 135)	p-value
Resistant to any two anti-TB drugs[a]	23	2	0.12	2	23	0.04
Multi-drug resistance[b]	40	4	0.001	2	42	0.16
Mono-resistance to any one anti TB drugs[c]	9	7	0.153	1	15	0.54

[a]Strains resistant to any two anti-TB drugs except a combination of isoniazid and rifampin; [b]Strains resistant to both isoniazid and rifampicin; [c]The anti-TB drugs tested were streptomycin, isoniazid, rifampin, and ethambutol.

The 24-locus and 15-locus noticeably improved the performance when compared with the primary 12-locus set, specifically in conjunction with spoligotyping. The 12-locus MIRU-VNTR typing alone produced 105 different patterns, and the combination of 12-locus MIRU-VNTR typing and spoligotyping increased to 111, with the HGDI values of 0.9898 and 0.991. Both 15-locus and 24-locus sets generated 137 genotypes and the combination of these two locus sets showed the same patterns. The HDGI values of 15-locus and 24-locus was equal (0.9996) and the combination of spoligotyping and these two locus sets was also the same.

The combination of 24-locus MIRU-VNTR and spoligotyping

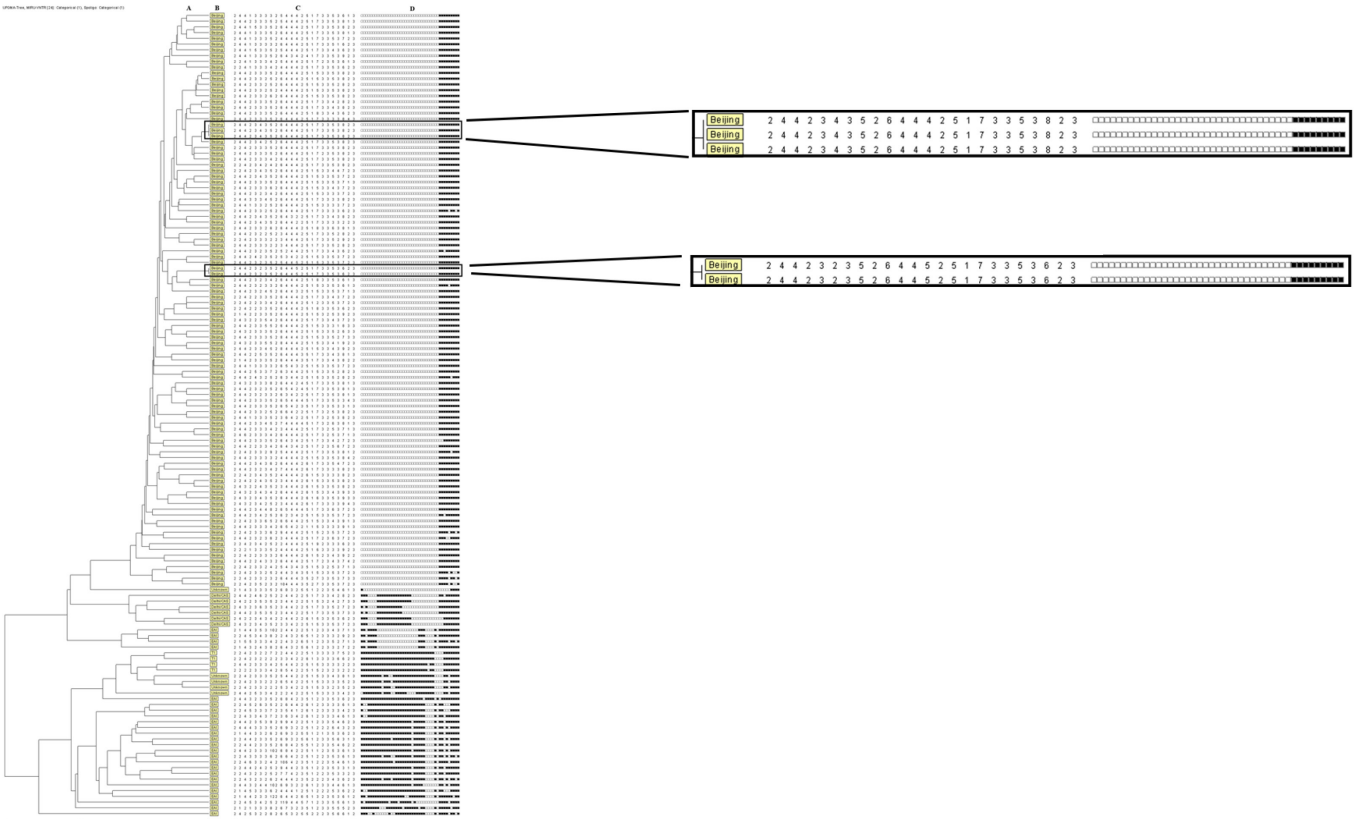
The dendogram of genetic relationship among MTB strains in Upper Myanmar could distinguish highly by using UPGMA (Unweighted paired group method with Arithmetic mean). All isolates were differentiated into 137 patterns including 2 clusters (clustering rate 3.6%) by combination of spoligotyping and 24-locus MIRU-VNTR (Figure 2).

Relationship between Beijing family and Drug resistance and demographic characteristics

Among all 140 isolates, 17.9% (25/140 isolates) were found to be resistant to any two anti-TB drugs except a combination of isoniazid and rifampin whereas 31.4% (44/140 isolates) were resistant to at least one of the first-line drugs (DR-TB). The percentage of drug resistance isolates was highly significant among patients infected with Beijing lineage isolates. Forty Beijing lineage isolates were resistant to both isoniazid and rifampicin and therefore, were defined as MDR-TB, while only 4 of 40 non-Beijing isolates were MDR (p-value = 0.04) (Table 4).

Then, the epidemiologic characteristics of Bejing and non-Beijing family are summarized in Table 5. The majority 64.3% of patients included were male while female were 35.7%. Beijing lineages were prevalent in both (15 to 24 and 25 to 34 years) (61/100 isolates). It

Figure 2. Spoligotyping and 24-loci MIRU-VNTR data of 140 M. tuberculosis isolates from Upper Myanmar.



(A) Dendrogram generated by UPGMA tree. (B) Isolates are identified according to their corresponding spoligotype international type (SIT), according to the SITVIT database. (C) MIRU-VNTR repeat numbers for 24 loci; the order of loci, left to right, is as follows: MIRU 02, Mtub 04, ETRC, MIRU 04, MIRU 40, MIRU 10, MIRU 16, Mtub 21, MIRU 20, Qub 216b, ETR A, Mtub 29, Mtub 30, ETR B, MIRU 23, MIRU 24, MIRU 26, MIRU 27, Mtub 34, MIRU 31, Mtub 39, Qub 26, Qub 4156, MIRU 39. (D) Spoligotyping profile. Two clonal clusters were found; three isolate cluster and two-isolate cluster (boxed).

can be assumed that Beijing strains were more likely prominent in young age (15 to 34 years) (*p*-value = 0.004).

Discussion

The genetic background of *M. tuberculosis* complex in Upper Myanmar alone is rarely known. The current study is the first insight in accessing genetic diversity of *M. tuberculosis* complex in Upper Myanmar using simultaneously two genotyping methods: 24-locus MIRU-VNTR typing and spoligotyping.

The predominant genotype found in this study was Beijing (71.4%) by combine two methods analysis which is highly prominent in Asian countries [21,22]. In a previous study, the EAI lineage was a first predominant and second was Beijing lineage [13,23]. The relationship of Beijing genotype and drug resistance may have a certain tendency for acquiring drug resistance which seems to be widely distributed (but not universal) [24]. In Myanmar, other previous studies have described that the Beijing genotypes in MDR and XDR populations are highly prevalent [25,26]. In the present study, the association between Beijing strains and MDR was highly significance (*p*-value < 0.001). Among Beijing isolates, 72 (51.4%) were infected with drug resistance isolates and 40 of them belonged to MDR. Two MDR strains were grouped into one cluster. Hence, Beijing strains in Upper Myanmar showed a greater correlation with Multi-drug resistance when compared with a previous study conducted in Yangon [13]. Furthermore, some studies revealed that Beijing genotypes is mostly associated with young age [27] and our study also proved that Beijing strains were prevalent in young age.

Although spoligotyping was successfully classified into 35 spoligotype patterns including 12 clusters and 23 unique patterns, it is a low resolution with the clustering rate (88.6%) and thus we performed MIRU-VNTR analysis. Based on the discriminatory power HGDI, a set of 15 loci was shown to have the same HGDI with 24-loci. In 24-locus MIRU-VNTR analysis, alleles Mtub 21, Qub 2163b, MIRU 26 and Qub 26 found high discriminatory power (HGDI ≥ 0.6). Some studies in China and India have suggested that those loci have highly discriminatory power [28,29]. A recent study in Myanmar has shown that Mtub21 was moderately discriminant (0.3 < HDGI < 0.6) [30]. On the other hand, our study found that alleles MIRU 02, ETRC, MIRU 20, Mtub 29, MIRU 23, MIRU 24, MIRU 27, Mtub 34 and MIRU 39(HGDI≤0.3) showed as poorest discriminatory power. A previous study in Iran has found that MIRU 10 has high HDGI. However, it showed moderate discriminatory power in our study. HGDI of MIRU 20 and MIRU 02 were similar with the previous study from Iran [31]. The overall HGDI of total isolates in our study was 0.9996 which is similar to previous studies [20,29].

In the present study, while the clustering rate of spoligotyping was 96.9% for Beijing strains, 24 locus MIRU-VNTR typing largely reduced to 4.6%. The previous study showed that the clustering rate of Beijing strains using 24-locus MIRU-VNTR typing was nearly 80% compared with non-Beijing family implying a high transmission rate [32]. Higher percentage of clustering rate indicates possible related

Table 5. Demographic data of 140 isolates among Beijing and Non-Beijing strains from Upper Myanmar by using combined typing.

Factors	Whole N = 140 (%)	Beijing N = 100 (%)	Non-Beijing N = 40 (%)	p-value
Gender				
Female	50 (35.7)	36 (36.0)	14 (35.0)	0.911
Male	90 (64.3)	64 (64.0)	26 (65.0)	
Age				
15-24	36 (25.7)	29 (29.0)	7 (17.5)	**0.004**
25-34	37 (26.4)	32 (32.0)	5 (12.5)	
35-44	29 (20.7)	21 (21.0)	8 (20.0)	
45-54	21 (15.0)	9 (9.0)	12 (30.0)	
55-64	11 (7.9)	6 (6.0)	5 (12.5)	
>65	6 (4.3)	3 (3.0)	3 (7.5)	
Treated before				
Yes	67 (47.9)	49 (49.0)	18 (45.0)	0.682
No	63 (45.0)	43 (43.0)	20 (50.0)	
Unknown	10 (7.1)	8 (8.0)	2 (5.0)	
Site of disease				
Pulmonary	138 (98.6)	98 (98.0)	40 (100)	0.368
Extra-pulmonary	2 (1.4)	2 (2.0)	0 (0.0)	

strains that are likely to spread to other geographical areas. In our study, the combination of spoligotyping and 24-locus MIRU-VNTR typing produced a diverse pattern of strains and only two clusters were found. One cluster that included two MDR strains (Beijing genotype) seemed to have acquired transmission of a drug resistance strain. While tuberculosis is highly prevalent in Myanmar, the recent transmission rate in this study was 2.1%, 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 showed 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, which may indicate a higher possibility of recurrence in our study area. Our study has sampling limitations that the clustering proportion of the MTB might be underestimated within the short time period of the study especially for the Beijing strains. Additional studies with larger sample sizes are necessary to establish the genetic outlines of MTB strains in order to provide a better understanding of transmissions dynamics of TB to differentiate a genetic relationship, clustering, and possible transmission rate in the future.

Conclusion

In conclusion, our study revealed a wide diversity of strains and transmission dynamics of some strains, which can provide a broader understanding of TB outbreaks and epidemiology in Upper Myanmar. MIRU-VNTR typing in conjunction with spoligotyping can efficiently analyze the epidemiological characteristics of MTB complex in this area. A several new spoligotypes including ST 265, ST 190, ST 1651, ST 1940, ST 89, ST 11, ST 292, ST 1506, ST 1409, and ST 167 were found in this study. Both 15 and 24-locus MIRU-VNTR typing showed similar discriminatory power (HGDI 0.9996). High discriminatory power of Mtub21, Qub2163b, MIRU 26, QUB 26 ($h \geq 0.6$) can be applied as the first line locus for future studies. The predominance of Beijing strains may be due to human interaction with foreign countries such as China, India and Bangladesh. Beijing lineages isolates were correlated with multi-drug-resistant phenotypes when compared with other lineage isolates. The lower clustering rate in our study indicates that acquired transmission occurred in this study period. The genotypic pattern suggests that the lower transmission rate may be due to a higher possibility of reactivation cases in Upper Myanmar. Additional studies with larger sample sizes are necessary to establish the genetic outlines of MTB strains in order to provide a better understanding of transmissions dynamics of TB.

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Ethical approval

This study protocol was approved by both Ethics Review Committee of the Department of Medical Research, Yangon, Myanmar (Ethics/DMR/2017/122) and Khon Kaen University Ethics Committee in Human Research, Khon Kaen, Thailand (Ethics number HE602220).

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