**Genotypic diversity of multidrug-, quinolone- and extensively drug-resistant
Mycobacterium tuberculosis isolates in Thailand**

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Abstract

Drug-resistant tuberculosis (TB), which includes multidrug-resistant (MDR-TB), quinolone-resistant (QR-TB) and extensively drug-resistant tuberculosis (XDR-TB), is a serious threat to TB control. We aimed to characterize the genotypic diversity of drug-resistant TB clinical isolates collected in Thailand to establish whether the emergence of drug-resistant TB is attributable to transmitted resistance or acquired resistance. We constructed the first molecular phylogeny of MDR-TB (n=95), QR-TB (n=69) and XDR-TB (n=28) in Thailand based on spoligotyping and proposed 24-locus multilocus variable-number of tandem repeat analysis (MLVA). Clustering analysis was performed using the unweighted pair group method with arithmetic mean. Spoligotyping identified the Beijing strain (SIT1) as the most predominant genotype (n = 139; 72.4%). The discriminatory power of 0.9235 Hunter-Gaston Discrimination Index (HGDI) with the 15-locus variable-number tandem repeats of mycobacterial interspersed repetitive units typing was improved to a 0.9574 HGDI with proposed 24-locus MLVA, thereby resulting in the subdivision of a large cluster of Beijing strains (SIT1) into 17 subclusters. We identified the spread of drug-resistant TB clones caused by three different MLVA types in the Beijing strain (SIT1) and a specific clone of XDR-TB caused by a rare genotype, the Manu-ancestor strain (SIT523). Overall, 49.5% of all isolates were clustered. These findings suggest that a remarkable transmission of drug-resistant TB occurred in Thailand. The remaining 50% of drug-resistant TB isolates were unique genotypes, which may have arisen from the individual acquisition of drug resistance. Our results suggest that transmitted and acquired resistance have played an equal role in the emergence of

drug-resistant TB. Further characterization of whole genome sequences of clonal strains could help to elucidate the mycobacterial genetic factors relevant for drug resistance, transmissibility and virulence.

Keywords: multidrug resistance; extensively drug-resistant; tuberculosis; MLVA; genotypic diversity; Thailand

1. Introduction

Despite improvements in tuberculosis (TB) treatment, with a success rate among all new TB cases reaching 86%, only 48% of multidrug-resistant tuberculosis (MDR-TB) patients are treated successfully (World Health Organization, 2014). The poor outcome of this treatment contributes to an increase in the mortality rate and leads to a greater risk of developing extensively drug-resistant tuberculosis (XDR-TB), which is considerably more difficult to treat. The World Health Organization classified the burden of MDR-TB and XDR-TB as a public health crisis. In Thailand, MDR-TB accounts for 2% and 19% of new TB and retreatment cases, respectively (World Health Organization, 2014). We analyzed data from routine second-line drug susceptibility testing of MDR-TB between 2003 and 2011. During this eight years period, quinolone-resistant tuberculosis (QR-TB), which is one form of pre-XDR-TB, increased from 9% to 15% of MDR-TB isolates, while XDR-TB isolates, which are resistant to quinolone and a second-line injectable drug, comprised 4% of MDR-TB isolates (Chaiprasert et al., 2014). Elucidation of the factors driving the emergence of drug-resistant TB is required to optimize strategies for TB control.

Epidemiological investigation is the first-line approach in the identification of the emergence of an infectious disease. However, it does not provide sufficient information about the mechanisms of emergence. The emergence of drug-resistant TB is complicated by two potential mechanisms: (i) transmission of drug resistance from a drug-resistant strain (transmitted resistance) and (ii) acquired drug resistance by a drug-susceptible strain (acquired resistance) (Blower et al., 2004; Klopper et al., 2013). Molecular

epidemiology has been used to clarify the cause of emerging drug-resistant TB in the community. The most notable application was the use of IS6110-based restriction fragment length polymorphism genotyping in the investigation of the emergence of MDR-TB in New York City from 1995 to 2001, which resulted in the discovery of the transmission of a specific strain of the W/Beijing genotype (Frieden et al., 1995; Munsiff et al., 2002).

A number of genotyping methods have been used to provide information concerning the genetic diversity among drug-resistant strains. Currently, multilocus variable-number of tandem repeat analysis (MLVA) is accepted as a standard polymerase chain reaction (PCR)-based method that is used in molecular epidemiology studies of TB, because it facilitates rapid analysis and provides digital data, which enable the comparison of data between laboratories (Mazars et al., 2000). MLVA data have been used in combination with classical epidemiologic data to provide detailed information on the relative proportion of acquired resistance versus transmitted resistance based on the genotypic diversity over time (de Beer et al., 2014; Marais et al., 2013; Shamputa et al., 2010).

In general, 15-locus variable-number tandem repeats of mycobacterial interspersed repetitive units (MIRU-VNTR) and 24-locus MIRU-VNTR are standard methods used in routine epidemiologic investigations and phylogenetic analysis, respectively (Supply et al., 2006). However, these techniques have the disadvantage of low discriminatory power between the Beijing family of *Mycobacterium tuberculosis* strains (Allix-Béguec et al., 2014; Gurjav et al., 2014), which is the most prevalent genotype in Thailand (Faksri et al., 2011; Park et al., 2000; Prodinger et al., 2001; Srilohasin et al., 2014). Hypervariable loci have been proposed to improve the efficiency of subtyping Beijing clusters. In this

study, we selected nine additional loci, which are described as highly polymorphic loci with allelic diversity among Beijing strains above 0.5 in previous reports (Allix-Béguec et al., 2014; Iwamoto et al., 2007; Murase et al., 2008).

Currently, genotyping of *M. tuberculosis* is not routinely performed in Thailand. Therefore, information about emerging drug-resistant TB is based predominantly on classical epidemiologic data without corresponding data informed by genetics (Jirapongsa et al., 2012; Lapphra et al., 2013; Punnotok et al., 2000). In this study, we aimed to characterize the genotypic diversity of drug-resistant TB clinical isolates (including MDR-TB, QR-TB and XDR-TB) collected in Thailand to establish whether the ongoing problem of drug-resistant TB is caused by the transmission of predominant clones or the emergence of acquired resistance. Spoligotyping, a simple and rapid method for detecting polymorphisms within the direct repeat locus, was used as a first-line genotyping tool. Furthermore, a proposed 24-locus MLVA was used for further subtyping of drug-resistant *M. tuberculosis* isolates. The discriminatory power of proposed 24-locus MLVA was evaluated in comparison with the standard 15-locus MIRU-VNTR to determine the effect of the nine additional loci on the subtyping of Beijing strains.

2. Materials and Methods

2.1. *Mycobacteria and drug susceptibility testing*

Two hundred and ten *M. tuberculosis* isolates from 196 TB patients were retrieved from stock cultures of clinical isolates deposited at the Drug-Resistant Tuberculosis Research Fund, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University from December 2003 to March 2013. The mycobacterial collection consisted of isolates from thirty provinces in all six geographical regions of Thailand. All isolates were obtained from decontaminated sputum cultured on Löwenstein-Jensen medium, followed by drug susceptibility testing using the proportion method on Middlebrook 7H10 agar quadrant plates. In total, 98 MDR-TB isolates, 82 QR-TB isolates and 30 XDR-TB isolates were successfully retrieved. The stock cultures were stored in Middlebrook 7H9 media with 15% glycerol at -20 °C. The study protocol was approved by the Ethical and Scientific Committees of the Faculty of Medicine Siriraj Hospital, Mahidol University (EC No. Si 029/2557).

2.2. *Genotyping of M. tuberculosis*

2.2.1. *Spoligotyping*

Genomic DNA was obtained by extraction using the cetyl-trimethyl-ammonium bromide-sodium chloride method, as previously described (van Soolingen et al., 1991). The whole sequence of the direct repeat (DR) region, including all spacers between the DR loci, was amplified by simplex PCR. The PCR amplicons were then used to perform

spoligotyping with a commercially available kit (Ocimum Biosolutions, India) as previously described (Kamerbeek et al., 1997). The binary spoligotype pattern was converted into a 15-digit octal code, which was later used to query the SITVIT WEB (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/) proprietary database of the Pasteur Institute of Guadeloupe for classification into clade and spoligotype international type (SIT) (Demay et al., 2012).

2.2.2. *Multilocus variable-number of tandem repeat analysis*

A set of standard 15-locus MIRU-VNTR (Supply et al., 2006), a consensus 4-locus set of hypervariable loci (1982, 3232, 3820 and 4120) (Allix-Béguec et al., 2014), a promising 4-locus set for subtyping Beijing family (2074, 2372, 3155 and 3336) (Murase et al., 2008) and a highly polymorphic locus (2163a) (Iwamoto et al., 2007) were amplified by multiplex PCR or a simplex PCR using 5' fluorescently labeled primers as shown in Supplementary Table S1. The multiplex PCR and simplex PCR master mixes were prepared as summarized in Supplementary Table S2 and S3, respectively. PCR reactions were performed under the following conditions: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 63°C for 30 s and extension at 72°C for 1.5 min, with a final extension at 72°C for 7 min. The size of the amplified PCR products was determined by capillary electrophoresis on a 3130 genetic analyzer or a 3500 genetic analyzer (Applied Biosystems, USA). The copy number of each locus was automatically estimated using GeneMapper[®] software v.5 (Applied Biosystems, USA). When an ambiguous peak or no peak was observed, the sample was subjected to re-analysis by simplex PCR with unlabelled primers. In this case, the size of

the PCR amplicon was determined by 2% agarose gel electrophoresis or capillary electrophoresis (SV1210 Microchip electrophoresis system, Hitachi High-Technologies Corporation, Japan), and the copy number of each locus was calculated manually.

2.3. Discriminatory power of genotypic methods and clustering analysis

The allelic diversity of each locus and the discriminatory power of the different genotypic methods were evaluated based on the *h*-value (Selander et al., 1986) and Hunter-Gaston Discriminatory Index (HGDI) (Hunter et al., 1988), respectively. For clustering analysis, the unweighted pair group method with arithmetic mean (UPGMA) was used to construct a rooted tree based on the categorical coefficients by Ridom MLVA Compare 1.05 (Ridom GmbH, Münster, Germany). A cluster was defined as two or more *M. tuberculosis* isolates with an identical genotype.

3. Results

3.1. Discriminatory power of genotypic methods

Sequential isolates with identical MLVA patterns that were taken from a single patient were counted as only one isolate for further analysis. Three isolates were excluded due to a lack of amplification of loci 2163a, 2163b and 2165, and one isolate was excluded due to mixed genotypes. Consequently, a total of 192 samples were used to determine the discriminatory power of the different genotypic methods. Spoligotyping had the lowest ability to differentiate *M. tuberculosis* genotype in our studied isolates. A standard 15-locus MIRU-VNTR offered a higher discriminatory power of 0.9235 HGDI among all isolates and 0.8683 HGDI among Beijing strains. A better resolution was obtained using 24-locus MLVA; as a result, discriminatory power among all isolates and Beijing strains were improved to 0.9574 HGDI and 0.9245 HGDI, respectively. The highest discriminatory power of 0.9577 HGDI among all isolates was provided by a combination of 24-locus MLVA and spoligotyping. A set of nine additional loci was superior to discriminate non-Beijing strains (0.9690 HGDI) than Beijing strains (0.9194 HGDI). Despite this, the maximum discriminatory power of 0.9834 HGDI among non-Beijing strains could be achieved with the standard 15-locus MIRU-VNTR (Table 1). As summarized in Table 2, five of the nine additional loci (loci 1982, 2163a, 3232, 3820 and 4120) showed high allelic diversity (h -value > 0.5) for the Beijing strains, although only one of the 15 standard loci (locus 2163b) showed high variability among the Beijing strains.

3.2. Spoligotyping-based clustering of isolates

Using spoligotyping, 174 isolates formed nine clusters, while the remaining 18 isolates had unique genotypes, thus resulting in a total of 27 spoligotypes. A comparison of the spoligotypes from the three different types of resistant strains revealed the marked differences in genotypic diversity among these three types (Table 3). In addition to the predominance of Beijing strain (SIT1) clone in MDR-TB (75.8%), QR-TB (73.9%) and XDR-TB (57.1%), the Manu-ancestor (SIT523) clone was the second most common genotype, accounting for 4.3% of QR-TB and an overrepresentation in XDR-TB at 39.2%, although it was absent from the MDR-TB group. In contrast, the T1 clade (SIT53) was the second most common clone in MDR-TB (4.2%). In addition, most of the unique spoligotypes that were found in MDR-TB were not found in QR-TB or XDR-TB. Three MDR-TB isolates were defined as orphan. Two of them exist in SITVIT WEB database as single isolates belonging to EAI5 and EAI6_BGD genotype. The remaining one did not match any existing spoligotype in the database.

3.3. MLVA-based clustering of isolates

Using 24-locus MLVA, the 192 isolates were divided into 115 MLVA types. A total of 95 isolates (49.5%) formed 18 different clusters, which ranged in size from two isolates to 36 isolates (Table 1). The remaining 97 isolates (50.5%) had unique genotypes. Three major MLVA clusters of the Beijing family (SIT1) were identified: cluster 4 (n = 37), 5 (n = 8) and 11 (n = 10) and were defined as predominant clones because these clusters were found in all three resistance types, were isolated from all regions of Thailand over the course of the study period and comprised 28.6% of all

isolates in this study. Intriguingly, the fourth largest cluster (cluster 18; $n = 6$) was specific to only XDR-TB with a distinct MLVA pattern typical of a Manu-ancestor (SIT523) clone (Table 4). Consequently, the isolates that were clustered among the three types of drug-resistant TB were categorized as belonging to two cluster types: (i) predominant clones and (ii) minor clusters. The relative proportion of predominant clones decreased from 32.6% ($n = 31/95$) of MDR-TB to 21.4% ($n = 6/28$) of XDR-TB, while the relative proportion of minor clusters increased from 14.7% ($n = 14/95$) of MDR-TB to 28.6% ($n = 8/28$) of XDR-TB. Approximately 50% of all types of drug-resistant TB were singletons (unique genotypes) (Table S4).

3.4. Clonal spread of MDR-TB and XDR-TB in Western Thailand

The combination of genotypic data and geographical information highlighted two instances of MDR-TB and XDR-TB spread in the vicinity of Kanchanaburi Province in Western Thailand. First, the largest clonal complex of MDR-TB, which comprised 15 isolates from a predominant clone, cluster 4, and two single locus variants, were detected sporadically during the period from 2006 to 2010 (Fig. 2A). Second, the clonal spread of XDR-TB with the specific MLVA type (Cluster 18) of Manu-ancestor (SIT523) was identified during the period from 2007 to 2012. Clonal complexes of this specific MLVA type and its single locus variants accounted for 25.0% (7 of 28 isolates) of the XDR-TB cases in this study (Fig. 2B). The partial map of Western part of Thailand is shown in figure 2C.

4. Discussion

The emergence of drug-resistant TB, including MDR-TB, QR-TB and XDR-TB, is continuously reported in Thailand (Chaiprasert et al., 2014; World Health Organization, 2014). Elucidation of the mechanisms underlying this problem is required for the development of appropriate control strategies. In this study, spoligotyping revealed the predominance of the Beijing genotype (SIT1), which accounted for the largest cluster (72.6%) among all the isolates. However, such a high percentage does not necessarily reflect an actual transmission event because of the poor discriminatory power of spoligotyping among Beijing strains, as shown in Table 1.

The present study confirmed that five of the additional loci (1982, 2163a, 3232, 3820 and 4120) contributed significantly to the subtyping of both Beijing and non-Beijing isolates as previous report (Allix-Béguec et al., 2014; Iwamoto et al., 2007; Murase et al., 2008). Although locus 2163a was excluded from the previous report due to amplification failure or an unreliably large amplification product, we included this locus because it represented characteristic high copy number of 18 copies (PCR product, approximately 1,400 bp) in two major clonal complexes of transmitted strains that caused clonal expansion of MDR-TB and XDR-TB. The remaining four loci (2074, 2372, 3155 and 3336) had low allelic diversity among the Beijing isolates, which is inconsistent with those of a previous study examining Japanese isolates (Murase et al., 2008). These discrepancies may be due to differences in the proportion of ancestral and modern Beijing strains. The percentage of ancestral Beijing strains is high in Japan (Iwamoto et al., 2009), whereas modern Beijing strains are more predominant in Thailand (Faksri et al.,

2011; Srilohasin et al., 2014). This hypothesis is supported by Luo et al. (2014), who found lower variability in VNTRs 2074 and 2372 from the ancestral Beijing strains than in the modern Beijing strains from China.

In this study, MLVA revealed a moderate level of genotypic diversity, which implicated composite mechanisms of resistance, including transmitted and acquired resistance, as a potential cause for the emergence of drug-resistant TB. In the present study, we have identified three predominant clones of the Beijing genotype (SIT1) as major causes of TB transmission. Many studies that examined the association between mycobacterial genetic background and transmission rate have demonstrated a higher transmission rate among Beijing strains than non-Beijing strains (Sun et al., 2007; van der Spuy et al., 2009; Yang et al., 2012). Despite this, variety exists among the Beijing strains. Hanekom et al. described differences among different Beijing sublineages in their ability to transmit and cause disease (Hanekom et al., 2007). This variation might explain why in this study we could distinguish the three different predominant clones of the Beijing strain (SIT1). In addition, we have observed that the predominant clones represented all three types of resistance. These data indicated that amplification of resistance to second-line drugs in MDR-TB may occur in those clones, thus leading to the development of QR-TB and XDR-TB from MDR-TB.

Surprisingly, we found that the fourth largest MLVA cluster of a rare SIT523 spoligotype accounted for 3.13% of all isolates in our study, whereas this spoligotype has been reported in only 27 of 58,180 global isolates (0.05%) and two of 307 Thai isolates (0.65%) in the SITVIT WEB database (Demay et al., 2012). The Manu-ancestor (SIT523) strain is considered to be a part of the Indo-Oceanic (lineage 1) (Shabbeer et al.,

2012). However, the minimum spanning tree based on MLVA data and whole genome SNP analysis demonstrated the connection between SIT523 and East Asian (lineage 2; includes Beijing family) (Blouin et al., 2012). Similarly, the minimum spanning tree created using MLVA profiles in our study also demonstrated that the SIT523 cluster was connected to cluster 4, the largest predominant clone of the Beijing genotype (SIT1) (data not shown), which might explain why SIT523 acquired efficient transmissibility. Interestingly, this cluster was only specific to XDR-TB. The dynamics of drug-resistant TB transmission vary depending on resistance-conferring mutations. Some specific mutations cause no fitness defect or compensate for fitness cost, for instance, *rpoB* S531L, *katG* S315T and compensatory mutations in *rpoA* and *rpoC*. These mutations are found to be associated with successful transmission (Casali et al., 2014; Gagneux, 2009). A distinct SIT523 strain may gain such mutations in its genome. Hence, our study emphasizes the problem of clonal expansion of a certain strain and the need for further examination of the transmissibility of this strain, which may contribute to the possibility of clonal expansion.

Approximately 50% of the isolates from each resistance group were unique genotypes (Fig. 1), which suggests that drug resistance developed in individual patients over the study period. There are limitations on interpretation of our results. The small and unequal sample size in each resistance group may affect the clustering analysis. In addition, geographic data is only available for predicting clonal expansions in this study; therefore, additional epidemiological and demographic data are needed to clarify the transmission. Furthermore, an adequate treatment history or investigation of drug susceptibility of sequential isolates for each patient is required to more precisely define

cases of acquired resistance. Despite these limitations, the selection of mycobacterial isolates was based on phenotypic resistance data only, without prior knowledge of the strain types; thus, the genotypic diversity observed is likely to reflect the true diversity among *M. tuberculosis* isolates in this study.

To our knowledge, this study provides the first insight into the genotypic diversity of drug-resistant TB in Thailand. Our results show the usefulness of genotypic diversity as a supportive tool for understanding the causes of emergence drug-resistant TB over study period. Firstly, the clonal spread of MDR-TB and XDR-TB provided the genetic evidence of previous MDR-TB community outbreak (Jirapongsa et al., 2012) and revealed a hidden spreading event of XDR-TB, respectively. Secondly, three major clusters among all resistance groups suggested the spread of predominant clones and amplification of resistance. Finally, a moderate number of unique genotypes hinted at possibility of acquired resistance as another factor driving the emergence of drug-resistant TB. The results of this study emphasize the need to strengthen TB control strategies and to adopt appropriate treatment regimens to prevent the further development of drug resistance. Additionally, a comprehensive study of whole genome sequences is required for understanding of the genetic factors that are relevant to the drug resistance, transmissibility and virulence of clonal strains.

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Legends

Table 1. Discriminatory power of spoligotyping and different multilocus variable-number of tandem repeat analysis schemes.

Table 2. Allelic diversity of each locus in the 24-locus MLVA scheme.

Table 3. Spoligotypes of multidrug-resistant (MDR), quinolone-resistant (QR) and extensively drug-resistant (XDR) tuberculosis.

Table 4. MLVA-based cluster details of drug-resistant TB isolates from all regions of Thailand from December 2003 to March 2013.

Fig. 1. Proportion of singletons, minor clusters and predominant clones among three different types of resistance. A singleton is a genotype appearing uniquely within the data set. Predominant clones are isolates in clusters 4, 5 and 11, consisting of all three types of drug-resistant TB, and are continuously isolated from all regions of Thailand. Minor clusters are the remaining clustered isolates. The numbers on each bar represent number of isolates in different categories; where n represent the total number of isolates of multidrug-resistant (MDR), quinolone-resistant (QR) and extensively drug-resistant (XDR) tuberculosis.

Fig. 2. Unweighted pair group method with arithmetic mean tree based on the 24-locus MLVA data of multidrug-resistant TB (A) and extensively drug-resistant TB (B) isolates from the vicinity of Kanchanaburi Province in Western Thailand. Boxes with dashed lines highlight the clonal complexes transmitted in this area. (C) Map of Kanchanaburi Province and vicinities: (1) Kanchanaburi, (2) Ratchaburi, (3) Suphanburi, (4) Bangkok and (5) Myanmar.

Table 1

Discriminatory power of spoligotyping and different multilocus variable-number of tandem repeat analysis schemes.

Methods	Clustered types	Unique types	Percentage of clustered isolates	Largest cluster size (%)	Hunter-Gaston Discriminatory Index (HGDI)		
					All isolates (n = 192)	Beijing (n = 143)	Non-Beijing (n = 49)
Spoligotyping	9	18	90.1%	139 (72.4)	0.4782	0.0551	0.8771
15-locus MIRU-VNTR	20	71	63.0%	48 (25.0)	0.9235	0.8683	0.9834
9 additional loci	21	83	56.8%	37 (19.3)	0.9546	0.9194	0.9690
15-locus MIRU-VNTR + 9 additional loci	18	97	49.5%	37 (19.3)	0.9574	0.9245	0.9834
Spoligotyping + 15-locus MIRU-VNTR + 9 additional loci	17	98	48.4%	37 (19.3)	0.9577	0.9253	0.9834

Table 2

Allelic diversity of each locus in the 24-locus MLVA scheme.

Locus	Unit (bp)	Copy no. of H37Rv	No. of alleles	Copy number	Allelic diversity (<i>h</i>) for: ^a		
					All isolates (n = 192)	Beijing (n = 143)	Non-Beijing (n = 49)
15-locus MIRU-VNTR							
2163b	69	5	9	1-9	0.785	0.699	0.842
4052	111	5	10	1-10	0.559	0.347	0.822
3690	58	5	8	1-7, 10	0.538	0.304	0.752
1955	57	1	10	1-6, 8-10, 18	0.534	0.357	0.790
2165	75	3	8	2-9	0.518	0.248	0.799
4156	59	3	5	0-4	0.505	0.287	0.468
0424	51	2	7	1-6, 9	0.476	0.297	0.664
0960	53	3	6	2-5, 8, 10	0.456	0.297	0.664
2996	51	3	8	1-3, 5-8	0.439	0.172	0.750
0580	53	2.5 ^b	11	0-9	0.387	0.221	0.697
2401	58	2	4	1-2, 4-5	0.366	0.041	0.280
1644	53	2	4	1-4	0.360	0.054	0.598
3192	53	3	7	2-8	0.336	0.081	0.710
0802	54	1	4	1-4	0.312	0.243	0.458
0577	58	4	4	2-5	0.041	0.000	0.155
9 additional loci							
3820	57	3	14	1, 3-5, 7-12, 14-17	0.831	0.765	0.863
2163a	69	2	11	2, 4-12, 18	0.791	0.741	0.856
3232	56	4	18	1, 4-20	0.779	0.639	0.788
4120	57	2	11	2-6, 8-13	0.734	0.574	0.725
1982	78	5	12	0, 2-12	0.685	0.524	0.774
2372	57	2	4	1-4	0.414	0.206	0.574
3336	59	8	9	4-12	0.391	0.172	0.742
2074	56	4	4	1-4	0.228	0.028	0.582
3155	54	4	5	2-6	0.193	0.146	0.319

^a Data was sorted in descending order of allelic diversity.

^b VNTR0580 of *M. tuberculosis* H37Rv possesses Type I repeat unit (77 bp), while most clinical isolates possess the Type II repeat unit (53 bp).

Table 3

Spoligotypes of multidrug-resistant (MDR), quinolone-resistant (QR) and extensively drug-resistant (XDR) tuberculosis.

	SIT ^a	Clades	No. of isolates (%)		
			MDR-TB (n = 95)	QR-TB (n = 69)	XDR-TB (n = 28)
Clustered Spoligotypes	1	Beijing	72 (75.8)	51 (73.9)	16 (57.1)
	48	EAI1_SOM	0	3 (4.3)	0
	50	H3	2 (2.1)	0	0
	53	T1	4 (4.2)	0	0
	236	EAI5	2 (2.1)	2 (2.9)	0
	246	Unknown	1 (1.1)	2 (2.9)	0
	523	Manu_ancestor	0	3 (4.3)	11 (39.3)
	591	EAI6_BGD	2 (2.1)	0	0
	941	Beijing	3 (3.2)	0	0
Unique Spoligotypes	35	H3	0	1 (1.4)	0
	89	EAI2_nonthaburi	1 (1.1)	0	0
	255	Beijing	0	1 (1.4)	0
	256	EAI5	0	1 (1.4)	0
	469	LAM1	0	0	1 (3.6)
	497	EAI5	1 (1.1)	0	0
	735	EAI1_SOM	0	1 (1.4)	0
	939	EAI5	0	1 (1.4)	0
	942	T2	0	1 (1.4)	0
	943	Ambiguous T2-T5	1 (1.1)	0	0
	955	Unknown	1 (1.1)	0	0
	962	EAI5	0	1 (1.4)	0
	1364	Beijing	1 (1.1)	0	0
	1378	Manu3	0	1 (1.4)	0
	2351	EAI2_Manila	1 (1.1)	0	0
	Orphan	EAI5	1 (1.1)	0	0
	Orphan	EAI6_BGD	1 (1.1)	0	0
	Orphan	-	1 (1.1)	0	0

^a SIT: Spoligotype International Type from SITVITWEB

Table 4

MLVA-based cluster details of drug-resistant TB isolates from all regions of Thailand from December 2003 to March 2013.

Cluster no.	Spoligotype	MLVA patterns ^a	No. of isolates	Type of resistance	Origin of isolates	Year of isolation
1	Beijing (SIT1)	4423334744753A4A3544F7CC	2	MDR	Eastern, Central	2009, 2010
2	Beijing (SIT1)	44233356447538283834D7BA	4	MDR	Northeastern, Southern	2007, 2012
3	Beijing (SIT1)	44233356447538283834G7EA	3	MDR, QR	Northeastern, Central	2009
4	Beijing (SIT1)	44233354447538283I34E79A	37	MDR, QR, XDR	All regions	2004–2012
5	Beijing (SIT1)	44233354447538283I34E799	8	MDR, QR, XDR	Northern, Eastern, Central, Western, Southern	2005, 2007–2010
6	Beijing (SIT1)	34233356447539083834E7BA	4	MDR, QR	Northern, Northeastern, Central	2005, 2007, 2012, 2013
7	Beijing (SIT1)	44223354447538283834F9CD	2	MDR, QR	Western, Central	2007, 2010
8	Beijing (SIT1)	44223335447537263834F7EA	2	QR	Eastern, Central	2006
9	Beijing (SIT1)	44233345347548283534I7H8	3	MDR	Southern	2009, 2010, 2012
10	Beijing (SIT1)	44033355447548263534E7EB	2	QR	Eastern, Western	2010, 2011
11	Beijing (SIT1)	44033355447548263534E7EA	10	MDR, QR, XDR	Northeastern, Eastern, Central, Southern	2004, 2006–2009, 2011–2012
12	Beijing (SIT1)	4403335544753826353477GC	2	QR, XDR	Central, Southern	2005, 2011
13	Beijing (SIT1)	24233354345538273634E7C9	2	MDR, XDR	Central, Southern	2008, 2010
14	Beijing (SIT1)	44232436549538283534F7CA	2	MDR, QR	Northeastern	2012
15	Beijing (SIT1)	342133464475383A38329AB8	2	QR	Central	2009
16	Beijing (SIT1)	34233344448539383832HA98	2	QR	Northeastern	2008, 2011
17	Beijing (SIT1)	44238245527418152I44CA83	2	QR	Northeastern	2008, 2012
18	Manu-ancestor (SIT523)	44238246527411152I34BAB2	6	XDR	Eastern, Western	2007 – 2008, 2012

^a Order of VNTR loci: VNTRs 0424, 0577, 0580, 0802, 0960, 1644, 1955, 2136b, 2165, 2401, 2996, 3192, 3690, 4052, 4156, 1982, 2074, 2163a, 2372, 3155, 3232, 3336, 3820 and 4120. The letters in the MLVA pattern represent a copy number higher than 9; the letter A represents 10 repeats; the letter B represents 11 repeats, and so on.

Figure 1

Figure 2a

Figure 2b

B

— 0.1

Figure 2c