Tuberculosis 114 (2019) 42–46

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: www.elsevier.com/locate/tube

Molecular Aspects

Association of *ubiA* mutations and high-level of ethambutol resistance among *Mycobacterium tuberculosis* Thai clinical isolates

Orawan Tulyaprawat[a], Angkana Chaiprasert[a,b], Piriyaporn Chongtrakool[a], Kamol Suwannakarn[a], Popchai Ngamskulrungroj[a,*]

[a] Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[b] Drug-Resistant Tuberculosis Research Fund, Siriraj Foundation, Bangkok, Thailand

ARTICLE INFO

Keywords:
ubiA mutation
Ethambutol resistance
M. tuberculosis
Genotype
Thailand

ABSTRACT

Ethambutol (EMB) is the first-line antituberculosis drug and a potential supplementary agent for a treatment regimen of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB). It has long been known that mutations in *embCAB* operon, encoding EMB target, arabinosyltransferase, confer resistance to EMB. Recently, *ubiA* was additionally reported to be specifically associated with high-level EMB resistance in *Mycobacterium tuberculosis*. However, such information on *ubiA* is very limited. This study aimed to investigate correlations between mutations in *ubiA* and phenotypic EMB resistance among EMB-resistant (EMB^R) *M. tuberculosis* Thai clinical isolates. Minimum inhibitory concentration (MIC) level of EMB and *ubiA* sequences were determined and analyzed. Of 68 EMB^R-MDR isolates, 8.9% harbored mutations in *ubiA*. However, 10.0% and 46.6% of EMB-sensitive (EMB^S)-MDR and pan-susceptible isolates also had *ubiA* mutations detected, respectively. Most nonsynonymous mutations, L31P, A35S, and V55M were only found in the EMB^R-MDR isolates except E149D which was also found in EMB^S-MDR and pan-susceptible isolates. A further phylogenetic analysis based on spoligotyping and IS*6110*-RFLP illustrated that E149D was in fact associated to EAI-families rather than EMB resistance. By excluding synonymous mutations and the E149D, we found a high correlation between *ubiA* mutations and high-level of EMB resistance with 100.0% specificity. In conclusion, despite its rare occurrence, mutations in *ubiA* can potentially be a marker for a detection of high level of EMB resistance at least in the MDR *M. tuberculosis* background.

1. Introduction

Ethambutol (EMB) is an important front-line antituberculosis drug and potential supplementary agent for a treatment regimen of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) [1]. EMB targets arabinosyltransferase (or the EmbCAB protein) encoded by the *embCAB* operon. EmbCAB protein plays a crucial role in polymerization of arabinose to arabinan, a component of the cell wall in *Mycobacterium tuberculosis* [2]. EMB acts as an arabinose analogue and binds to EmbCAB protein causing arrest of the arabinan biosynthesis and interrupt the formation of the mycolyl–arabinogalactan–peptidoglycan complex. This leads to the accumulation of mycolic acid and the loss of membrane permeability, eventually leading to cell death [3,4]. Allelic exchanged studies proved that mutations in the *embCAB* confer resistance to EMB [5]. However, several studies found that *M. tuberculosis* clinical isolates could still resist to EMB without any mutations detected in *embCAB* [6–8]. On the other hand, mutations in *embCAB* among EMB susceptible clinical isolates were also found [9,10]. These suggested that additional mechanisms might facilitate *M. tuberculosis* resistance to EMB.

In 2013, Safi et al. proposed that mutations in another gene, *ubiA* (*Rv3806c*), resulted in high-level resistance to EMB [2]. *ubiA* encodes DPPR (decaprenylphosphoryl-β-D-5-phosphoribose) synthase involving in DPA (decaprenylphosphoryl-β-D-arabinose) synthesis. Typically, EmbCAB protein cleaves arabinose out of DPA to form arabinan, a crucial component in mycobacterial cell wall. Mutations in *ubiA* lead to an increase in DPA level. Subsequently, the increased intracellular DPA competitively binds to EmbCAB protein with EMB resulting in the high level of the drug resistance. Moreover, a previous study demonstrated that *ubiA* mutations only occurred in EMB resistant clinical isolates [11]

* Corresponding author. Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Rd, Bangkoknoi, Bangkok, 10700, Thailand.
E-mail addresses: orawan.typw@gmail.com (O. Tulyaprawat), angkana.cha@mahidol.ac.th (A. Chaiprasert), piriyaporn.cho@mahidol.ac.th (P. Chongtrakool), kamol.suw@mahidol.ac.th (K. Suwannakarn), popchai.nga@mahidol.ac.th (P. Ngamskulrungroj).

https://doi.org/10.1016/j.tube.2018.11.006
Received 7 September 2018; Received in revised form 12 November 2018; Accepted 17 November 2018

and prevalence of *ubiA* mutation varied depending on geographic origins [12]. However, despite its first report in 2013, information on specific correlation between *ubiA* mutations and the high EMB resistance level was limited [2]. Therefore, this study aimed to investigate *ubiA* mutations in EMB-resistant and EMB-susceptible *M. tuberculosis* Thai clinical isolates and their associations with the level of EMB resistance.

2. Materials and methods

2.1. Mycobacterial strains and anti-TB drug susceptibility testing

Mycobacterial strains were recovered from a −80 °C culture collection of the Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. All EMB-resistant *M. tuberculosis* clinical isolates between 2012 and 2015 were included in this study. On the other hand, strains which lost their viability and isolated from the same patients were excluded. All the recovered isolates were confirmed for isoniazid, rifampicin, streptomycin, and EMB susceptibility testing with proportional method on Middlebrook 7H10 agar, according to critical concentration recommended by the Clinical and Laboratory Standards Institute [13]. A sample size was calculated based on an estimated prevalence of *ubiA* mutation. However, prevalence of *ubiA* mutation among clinical isolates of EMB resistant *M. tuberculosis* was not previously reported in Thailand. Therefore, an average prevalence of *ubiA* mutation reported from other countries (China [11], Korea [12], India [14], and Africa [12]), of 20.13% was used. We defined confidence level of 95% and acceptable margin of error of 10%. The sample size was calculated by Cochran's formula according to a previous study [15] resulting in that at least 62 isolates were required. Isolates, lost the susceptibility pattern to the drugs, were not included. Finally, a total of 68 MDR (resisted to at least isoniazid and rifampicin) EMB-resistant isolates (EMB^R-MDR) were collected and analyzed. Furthermore, 33 MDR ethambutol-susceptible isolates (EMB^S-MDR) and 33 pan-susceptible isolates (susceptible to isoniazid, rifampicin, ethambutol, and streptomycin) were used as controls. EMB susceptible isolates were considered when MIC was less than or equal to 5 μg/ml EMB (Sigma-Aldrich, St. Louis, MO, USA), while the resistant isolates were considered when MIC was more than 5 μg/ml EMB. Low- and high-level resistances were also determined according to a previous study [11]. However, unlike our study, Löwenstein-Jensen (LJ) medium was used in the previous study [11]. Cut points for the low- and high-level resistance was adjusted for Middlebrook 7H10 according to the WHO guideline [16]. Therefore, the resistant isolates were determined as low-level resistant if their MIC was more than 5 μg/ml but less than or equal to 10 μg/ml EMB, and as high-level resistant if the MIC was more than 10 μg/ml EMB [11,16]. This study was approved by the Siriraj Hospital Ethics Committee (COA number: Si 504/2014).

Table 1
Distribution of *ubiA* mutations in clinical isolates of *M. tuberculosis* from Thailand during 2012 and 2015.

ubiA(Rv3806c) **mutation**		**Number of isolates**		
Amino acid alteration	**Nucleotide change**	**EMB^R-MDR**	**EMB^S-MDR**	**Pan-susceptible**
Wild type	**Wild type**	**62 (91.1%)**	**27 (90.0%)**	**16 (53.4%)**
Variant		**6 (8.9%)**	**3 (10.0%)**	**14 (46.6%)**
L31P*	C**T**G 31 C**C**G	1 (1.5%)		
A35S*	**G**CG 35 **T**CG	2 (2.9%)		
V55M*	**G**TG 55 **A**TG	1 (1.5%)		
E149D*	GA**A** 149 GA**C**			13 (43.3%)
E149D*, R76R	GA**A** 149 GA**C**, CG**T** 76 CG**C**	1 (1.5%)	3 (10.0%)	
V153V	GT**G** 153 GT**A**			1 (3.3%)
T168T	AC**C** 168 AC**G**	1 (1.5%)		
Total		**68 (100.0%)**	**30 (100.0%)**	**30 (100.0%)**

Notes: *nonsynonymous mutations.
Abbreviations: EMB^R-MDR, ethambutol-resistant with multidrug resistance; EMB^S-MDR, ethambutol-susceptible with multidrug resistance.

2.2. Genomic DNA isolation from M. tuberculosis

Genomic DNA was obtained from 3–4-week-old *M. tuberculosis* colonies cultured on LJ medium. The bacterial cells were suspended in 400 μl of TE (10 mM Tris-HCl, 10 mM EDTA; pH 8.0) buffer and then killed at 80 °C for 20 min. The bacterial cell walls were then lysed with 50 μl of 10 mg/ml lysozyme at 37 °C overnight. Subsequently, 75 μl containing of 10% SDS and 20 mg/ml proteinase K were added and mixed gently prior to incubation at 65 °C for 10 min. The cell wall debris and other impurities were removed by the CTAB/chloroform–isoamyl alcohol protocol. The genomic DNA was then precipitated with isopropanol and washed once with 70% alcohol. The genomic DNA pellet was redissolved in distilled water. Finally, the isolated genomic DNA was quantified and assessed for purity with a NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and stored at −20 °C until use.

2.3. PCR amplification and detection of ubiA mutations

The forward primer (ubiA-F; 5′-ACGTTGAGCTTGAGGCTAGC-3′) and reverse primer (ubiA-R 5′-CGCTGTCGCGAATACTGCT-3′) were used for the full-length of *ubiA* amplification as previously described [12]. The PCR reaction was performed in a volume of 50 μl containing, 1X *Taq* buffer supplemented with KCl, 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 μM ubiA-F and 0.2 μM ubiA-R primers, 100 ng of genomic DNA, and 1.25 U of *Taq* DNA polymerase (Thermo Scientific, Lithuania). The reaction was performed in thermal cycler starting with initial denaturation at 95 °C for 3 min. Subsequently, it was run through 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1.30 min, followed by final extension at 72 °C for 5 min. After the amplification, PCR product was purified with GeneJET™ PCR Purification Kit (Thermo Scientific, Lithuania) and sequenced directly by Macrogen Inc., Korea using 23 ABI 3730XLs DNA sequencing technology (Applied Biosystem, Foster City, USA) and BigDye Taq FS terminators. To identify *ubiA* mutations, all sequences were compared to the reference sequence of *M. tuberculosis* H37Rv (GenBank accession no. **NC000962.3**) using the MEGA 7.0 software (http://www.megasoftware.net/). Any nucleotides that differed from the reference sequence were defined as point mutations.

2.4. Spoligotyping

Spoligotyping was performed according to the manufacturer's instructions (Mapmygenome, India) [17]. Briefly, polymorphic of non-repetitive spacers were amplified by using biotinylated DRa (5′-biotin-GGTTTTGGGTCTGACGAC-3′) and DRb primer (5′-CCGAGAGGGGACG GAAAC-3′). The amplified products were detected for presence or absence of 43 spacers by hybridizing them to nylon membrane immobilized with each spacer probe. Then, the amplified products that bound to spacer probes were visualized by the enhanced chemiluminescent (ECL) detection system. Positive and negative signal was represented by 1 and 0, respectively, and later converted into octal code designation [17].

The octal code format of each strain was entered to assign Spoligotype International Type (SIT) and phylogenetic clade by using SITVITWEB, a database of the Pasteur Institute of Guadeloupe (http://www.pasteur-guadeloupe.fr:8081/SITVITONLINE/) [18]. A spoligotype reported with a single isolate was defined as orphan, while the pattern which had not been described in the database was defined as new SIT. Clade assignment of new SIT strain was achieved based on SpotClust database (http://tbinsight.cs.rpi.edu/about_spotclust.html), as previously described [19].

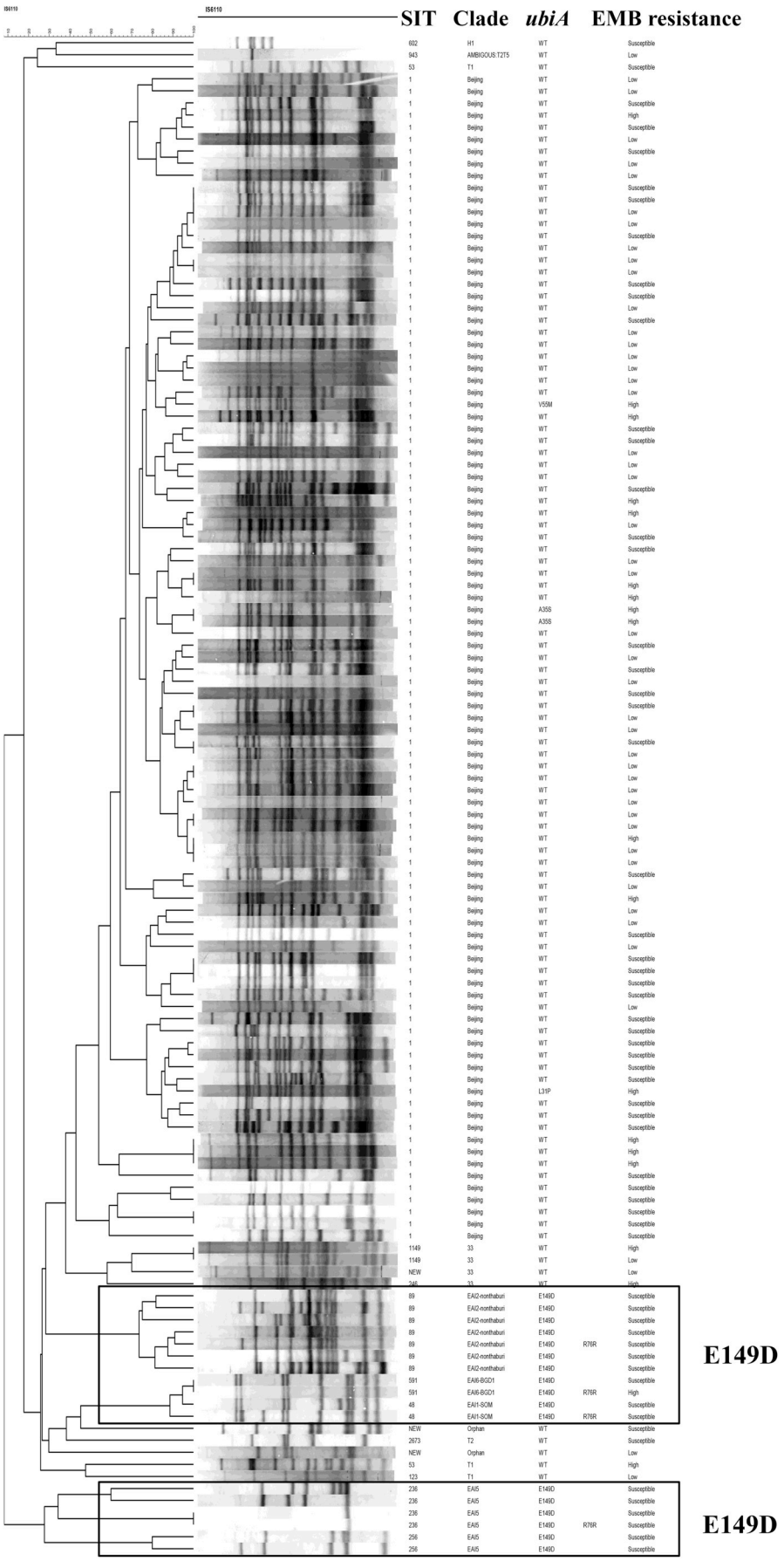

Fig. 1. Analysis of spoligotyping and IS*6110*-RFLP based phylograms with *ubiA* mutations and phenotypic EMB resistance. **Abbreviations:** SIT, Spoligotype International Type; EMB, ethambutol.

Table 2
ubiA mutations and level of EMB resistance in *M. tuberculosis* Thai clinical isolates.

ubiA (*Rv3806c*) mutation	Number of isolates with various level of EMB resistance	
	Low-level	High-level
Wild type, synonymous, or lineage specific	48 (100.0%)	16 (80.0%)
Nonsynonymous	0 (0.0%)	4 (20.0%)
Total	**48 (100.0%)**	**20 (100.0%)**

2.5. IS6110-RFLP

Genetic diversity of all isolates were analyzed by IS*6110*-RFLP followed a standardized procedure [20]. To visualize the IS*6110* fingerprint patterns, 4 μg genomic DNA isolated from *M. tuberculosis* was digested at 37 °C for 3 h by 5 unit of *Pvu*II (New England Biolabs, UK), which cleaves once in IS*6110* sequence. Then, the fragmented DNA was separated by running on 1% agarose gel and transferred onto a nylon membrane. The DNA was immobilized to the membrane by UV cross-linking, followed by hybridizing with DIG-labelled DNA probes. Finally, DIG labelled probe/DNA hybrids were detected by colour development. The IS*6110* banding patterns were analyzed by Fingerprinting II Informatix™ Software (Bio-Rad Laboratories, California, USA). The IS*6110*-RFLP profiles of strains were compared and clustered by grouping analysis with Dice coefficient and the unweighted pair group method with averages (UPGMA). The constructed phylogenetic tree had a genotypic clade designated according to spoligotyping. The genotypic clades were then determined for their associations with the *ubiA* mutation and EMB susceptibility.

2.6. Statistical analysis

Sensitivity and specificity were calculated to determine a detection power of EMB resistance level by *ubiA* mutations.

3. Results

3.1. ubiA mutations were detected in both EMB-susceptible and EMB-resistant M. tuberculosis in Thai clinical isolates

To determine the association between *ubiA* mutations and EMB resistance, 68 isolates of EMB^R-MDR were collected from the Department of Microbiology, Faculty of Medicine Siriraj Hospital during 2012 and 2015, and 30 EMB^S-MDR and 30 pan-susceptible clinical isolates were included as controls. EMB-monoresistant isolate was very rare and not present in the time of collection. Therefore, none of the isolate was included in this study. A nucleotide sequence analysis found that 8.9% of EMB^R-MDR isolates contained mutations in *ubiA*. Surprisingly, 10.0% of the EMB^S-MDR and 46.6% of the pan-susceptible isolates also had their *ubiA* mutations detected. However, almost all nonsynonymous mutations in *ubiA*, L31P, A35S, and V55M were unique to the EMB^R isolates except E149D which was presented in EMB^R, EMB^S and pan-susceptible isolates, as shown in Table 1.

3.2. Spoligotyping and IS6110-RFLP analysis revealed that the mutation at E149D might be lineage-specific mutation

The controversial, E149D mutations, which were detected in both EMB^R and EMB^S isolates might be lineage-specific. To test this hypothesis, two genotyping approaches, spoligotyping and *IS6110*-RFLP, were performed. The phylogenetic analysis illustrated that strains with E149D was linked to isolates in East African-Indian (EAI) families (EAI2-nonthaburi, EAI6-BGD1, EAI1-SOM, and EAI5) (Fig. 1).

3.3. Association between ubiA mutations and level of EMB resistance

Safi et al. demonstrated that *ubiA* mutations conferred high level EMB resistance [2]. Therefore, we investigated association between *ubiA* mutation and the level of EMB resistance. It was obvious that *ubiA* mutations potentially conferred high-level resistance, as it was only detected in the high-level EMB^R-MDR isolates (Table 2). The sensitivity and specificity of *ubiA* mutations for detection of EMB resistance level were 20.0% and 100.0%, respectively.

4. Discussion

This study showed overall prevalence of *ubiA* mutations among EMB-resistant *M. tuberculosis* clinical isolates in Thailand (8.9%) was comparable to that of China (8.3%) and South Korea (9.5%) [11,12]. In contrast, the prevalence of *ubiA* mutations was reported to be higher in North India (17.2%) and Africa (45.5%) [12,14]. It was observed that East Asia, which is an endemic area of Beijing genotype (Lineage2), generally had a low prevalence of *ubiA* mutations. On the other hand, regions with low prevalence of the Beijing genotype, such as India and Africa, had a high prevalence of *ubiA* mutations in EMB^R isolates [12,14]. As Thailand was an endemic area of the Beijing genotype, low prevalence of the *ubiA* mutations was likely and was evident in our study.

Similarly, the high correlation between *ubiA* mutation and EMB resistance in this study could be masked by lineage-specific mutations. For example, E149D was in fact related to the EAI lineage not to EMB resistance. In addition, several studies also detected E149D in both EMB^R and EMB^S isolate [2,11,14]. The association between E149D and the lineage of *M. tuberculosis* was also predicted previously by a comparative genome analysis [21]. By excluding synonymous mutations and the lineage-specific E149D, we found high association between *ubiA* mutations and high-level of EMB resistance with 100.0% specificity. Our result supported the contribution of *ubiA* mutations to high level EMB resistance as suggested in the previous study [2].

The present study discovered novel mutations in *ubiA*, including L31P, A35S and V55M. These three mutations located in the first transmembrane domain of UbiA protein [12]. In fact, mutation at *ubiA* codon 35 (A35E) was previously detected in *in vitro* selected EMB-resistant strain, while mutation at *ubiA* codon 55 (V55G) was recently found in EMB-resistant clinical isolate [12]. However, of the three novel mutations, L31P, A35S and V55M, ability to confer EMB resistance is yet to be confirmed by further isolates sampling or allelic exchange studies. A previous finding demonstrated that strains from Beijing lineage acquired resistance to anti-TB drugs more rapidly than strains from EAI-lineage [22]. Some studies reported that Beijing strains had higher mutation frequency in rifampicin and streptomycin target genes compared to non-Beijing strains [22,23]. This might lead to the rapid mutations occurred in Beijing strains in response to the drug stress environment. However, the association between Beijing lineage and mutations conferring anti-TB resistance is still required further investigation.

According to the low prevalence of *ubiA* mutations in Thai clinical isolates, the use of *ubiA* mutations for prediction of resistance level of EMB is limited as shown by its low sensitivity (20.0%). The specificity and sensitivity figures was somewhat similar to a previous study with 98.6% specificity and 8.1% sensitivity [11]. Considering figures from both our study and the previous study, the fact that there was 80–91.9% of isolates, resisting to EMB in high level without any mutations in *ubiA*, suggested additional unidentified genes or mechanisms involving in high level of EMB resistance in *M. tuberculosis*.

5. Conclusion

Despite its low prevalence, the *ubiA* mutation is a potential marker for a high level of EMB resistance. However, as mono-resistance of EMB

was very rare and, therefore, absent in this study. Such assumption is limited to only MDR *M. tuberculosis* background.

Acknowledgment

This research project was supported by Faculty of Medicine Siriraj Hospital, Mahidol University [grant number (IO) R015932009].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2018.11.006.

References

[1] WHO. WHO treatment guidelines for drug-resistant tuberculosis 2016 update. World Health Organization; 2016.
[2] Safi H, Lingaraju S, Amin A, et al. Evolution of high-level ethambutol-resistant tuberculosis through interacting mutations in decaprenylphosphoryl-β-D-arabinose biosynthetic and utilization pathway genes. Nat Genet 2013;45(10):1190.
[3] Mikusova K, Slayden RA, Besra GS, Brennan PJ. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. Antimicrob Agents Chemother 1995;39(11):2484–9.
[4] Takayama K, Kilburn JO. Inhibition of synthesis of arabinogalactan by ethambutol in *Mycobacterium smegmatis*. Antimicrob Agents Chemother 1989;33(9):1493–9.
[5] Nebenzahl-Guimaraes H, Jacobson KR, Farhat MR, Murray MB. Systematic review of allelic exchange experiments aimed at identifying mutations that confer drug resistance in *Mycobacterium tuberculosis*. J Antimicrob Chemother 2013;69(2):331–42.
[6] Ramaswamy SV, Amin AG, Göksel S, et al. Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2000;44(2):326–36.
[7] Starks AM, Gumusboga A, Plikaytis BB, Shinnick TM, Posey JE. Mutations at *embB* codon 306 are an important molecular indicator of ethambutol resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2009;53(3):1061–6.
[8] Zhao L-l, Sun Q, Liu H-c, et al. Analysis of *embCAB* mutations associated with ethambutol resistance in multidrug-resistant *Mycobacterium tuberculosis* isolates from China. Antimicrob Agents Chemother 2015;59(4):2045–50.
[9] Mokrousov I, Otten T, Vyshnevskiy B, Narvskaya O. Detection of *embB306* mutations in ethambutol-susceptible clinical isolates of *Mycobacterium tuberculosis* from Northwestern Russia: implications for genotypic resistance testing. J Clin Microbiol 2002;40(10):3810–3.
[10] Shi R, Zhang J, Otomo K, Zhang G, Sugawara I. Lack of correlation between *embB* mutation and ethambutol MIC in *Mycobacterium tuberculosis* clinical isolates from China. Antimicrob Agents Chemother 2007;51(12):4515–7.
[11] Xu Y, Jia H, Huang H, Sun Z, Zhang Z. Mutations found in *embCAB, embR*, and *ubiA* genes of ethambutol-sensitive and-resistant *Mycobacterium tuberculosis* clinical isolates from China. BioMed Res Int 2015;2015.
[12] Lingaraju S, Rigouts L, Gupta A, et al. Geographic differences in the contribution of *ubiA* mutations to high-level ethambutol resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2016;60(7):4101–5.
[13] Desmond E. Susceptibility testing of mycobacteria, nocardiae and other aerobic actinomycetes. Clinical Laboratory Standard Institute; 2011. M24-A22.
[14] Giri A, Gupta S, Safi H, et al. Polymorphisms in *Rv3806c* (*ubiA*) and the upstream region of *embA* in relation to ethambutol resistance in clinical isolates of *Mycobacterium tuberculosis* from North India. Tuberculosis 2018;108:41–6.
[15] Daniel WW, Cross CL. Biostatistics: a foundation for analysis in the health sciences: a foundation for analysis in the health sciences. Wiley Global Education; 2012.
[16] WHO. Updated interim critical concentrations for first-line and second-line DST (as of May 2012). Geneva, Switzerland: World Health Organization; 2012.
[17] Driscoll JR. Spoligotyping for molecular epidemiology of the *Mycobacterium tuberculosis* complex. Molecular epidemiology of microorganisms. Springer; 2009. p. 117–28.
[18] Demay C, Liens B, Burguière T, et al. SITVITWEB–a publicly available international multimarker database for studying *Mycobacterium tuberculosis* genetic diversity and molecular epidemiology. Infect Genet Evol 2012;12(4):755–66.
[19] Shabbeer A, Ozcaglar C, Yener B, Bennett KP. Web tools for molecular epidemiology of tuberculosis. Infect Genet Evol 2012;12(4):767–81.
[20] Van Embden J, Cave MD, Crawford JT, et al. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol 1993;31(2):406–9.
[21] Galagan JE. Genomic insights into tuberculosis. Nat Rev Genet 2014;15(5):307–20.
[22] Ford CB, Shah RR, Maeda MK, et al. *Mycobacterium tuberculosis* mutation rate estimates from different lineages predict substantial differences in the emergence of drug-resistant tuberculosis. Nat Genet 2013;45(7):784.
[23] Sun Y-J, Luo J-T, Wong S-Y, Lee A. Analysis of *rpsL* and *rrs* mutations in Beijing and non-Beijing streptomycin-resistant *Mycobacterium tuberculosis* isolates from Singapore. Clin Microbiol Infect 2010;16(3):287–9.