Philippine Journal of Science
150 (2): 417-427, April 2021
ISSN 0031 - 7683
Date Received: 29 Jun 2020

Molecular Epidemiologic Analysis of *Mycobacterium tuberculosis* among Prison Inmates in Selected Prisons in the Philippines

Jaime C. Montoya[1]*, **Maridel P. Borja**[2], **Concepcion F. Ang**[1], **John Carlo M. Malabad**[1], and **Yoshiro Murase**[3]

[1]College of Medicine, University of the Philippines, Manila 1000 Philippines
[2]College of Public Health, University of the Philippines, Manila 1000 Philippines
[3]Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Tokyo, Japan

Tuberculosis (TB) remains an important public health problem in developing countries like the Philippines. The success of the National TB Control Program depends on a clear understanding of the dynamics of transmission and spread of TB in high-risk populations in the community. We conducted a molecular epidemiologic analysis of *M. tuberculosis* isolates collected from inmates with pulmonary TB in selected prisons in the Philippines. A total of 25 isolates were characterized and genotyped using Spoligotyping and 15-loci MIRU-VNTR (mycobacterial interspersed repetitive units–variable number of tandem repeats) typing. The majority of the patients were male (84%) and aged 30–49 yr old (68%). Eighteen (72%) of the culture-positive patients had severe pulmonary TB, 13 (52%) were smear-positive, and seven (28%) were classified as having a high bacillary load. Twenty isolates (80%) were susceptible to all the first-line drugs. Two (8%) were multidrug-resistant (MDR) and isolated from patients in the same prison, one of which was resistant to all first-line drugs. Three isolates (12%) were streptomycin-monoresistant. There were nine identified Spoligo-International Types (SITs), with SIT19 as the predominant (40%). One isolate (4%) did not match any SIT in the SpoIDB4 database, while three were not assessed due to inadequate DNA for analysis. The distribution of strains according to major *M. tuberculosis* clades were as follows: EAI2_Manilla (48%) > LAM2 (20%) > LAM6 (8%) = LAM9 (8%). Spoligotyping identified two clusters and 13 genotypes (four unique strains) with a Hunter-Gaston discriminatory index (HGDI) of 0.83. MIRU-VNTR typing identified two clusters and 23 genotypes (HGDI = 0.993). Combined Spoligotyping and MIRU-VNTR typing also identified two clusters and 23 genotypes (HGDI = 0.993). There were no significant associations shown among host demographic factors, severity of the disease, drug resistance, and *M. tuberculosis* strain. We conclude that our patient population was infected predominantly by *M. tuberculosis* belonging to the EAI2_Manila clade.

Keywords: *Mycobacterium tuberculosis*, molecular epidemiology, prison, tuberculosis

INTRODUCTION

TB remains a major cause of morbidity and mortality despite intensive global and country efforts. In 2018, there were an estimated 1.5 million people in the world who died of TB, with the majority of cases taking place in developing countries, where *Mycobacterium tuberculosis* transmission has been associated with factors like crowding and poor or weak public health infrastructure. The overlap of TB with the HIV pandemic and the emergence of MDR *M. tuberculosis* strains are projected to complicate current strategies for control, hence the need for evaluating the relative contribution of these factors

*Corresponding Author: jmontoya204@gmail.com

in the current global epidemiology of TB (WHO 2019).

The World Health Organization (WHO) Global TB Report 2019 ranks the Philippines as fourth in having the highest number of incident TB cases in the world – next to India, China, and Indonesia (WHO 2019). According to the 2016 National TB Prevalence Survey, around 760,000 Filipinos aged 15 yr and above have pulmonary TB. The estimated prevalence of bacteriologically confirmed PTB (*i.e.* positive for TB culture and/or Xpert MTB/RIF) in this age group was 1,159 per 100,000 population, and that of smear-positive PTB was 434 per 100,000 population (DOH 2017). Compliance with the prescribed drug regimen in TB patients is also generally low, thus increasing the risk for the development of MDR strains. The NTPS 2016 showed that the incidence of MDR-TB in the general population (non-HIV), defined as resistance to isoniazid and rifampicin with or without resistance to the other first-line drugs, was 2.6% (DOH 2017).

Although the rate of case finding of active TB in the Philippines has improved over the years due to joint efforts of the public and private sectors, challenges remain that may potentially reduce the gains achieved in the prevention and control of this public health disease. One of these is the great magnitude of the TB problem in high-risk populations like prisoners and inmates in correction facilities and penitentiaries. Rates among prison inmates remain much higher – up to 50 times – than those of national averages across both the developed and the developing world. Some of the reasons that may explain the high prevalence of TB in the setting of prisons are as follows: 1) a high proportion from population groups that are already at high risk for TB infection and diseases such as alcohol or drug users, mentally ill individuals, former prisoners, and illegal immigrants from areas with high TB prevalence; overcrowding; late case detection; inadequate treatment of infectious cases; high turnover of prisoners; and poor implementation of TB infection control measures (Ijaz *et al.* 2004). All these factors contribute to higher transmission rates of TB in correction facilities and penitentiaries.

A local study done in seven selected prisons documented that approximately 17.5/1000 prisoners and jail officers had bacteriologically-confirmed TB, almost three-fold higher compared to the prevalence in the general population (Borja *et al.* 2011). Despite the fact that many state prisons worldwide have repeatedly reported the high incidence of TB and drug-resistant TB, little or nothing is being done to detect or scientifically treat inmates with TB in some of these correctional facilities.

The high incidence rates of TB in prisons will eventually impact the incidence rates of TB outside of the penitentiary. Released prisoners can infect people in their communities constituting a significant public health risk. A biomolecular approach, which involves genotyping of *M. tuberculosis,* used in several countries has proven that TB transmission inside prisons contributes significantly to high TB incidence rates in prison populations (Ijaz *et al.* 2004). A variety of genotyping techniques have been successfully used in describing the molecular epidemiology of *M. tuberculosis*. Such tools have helped TB control programs to determine population-level risk factors for transmission, identify potential TB hot spots where significant transmission is occurring, and evaluate TB control programs and strategies (Mathema *et al*. 2006).

Genotyping of *M. tuberculosis* isolates may also be used to differentiate between recently transmitted and reactivation disease. In population-based studies, isolates that share the same genotype are considered clustered and are assumed to be epidemiologically linked, although the link may be indirect. By contrast, cases with isolates of a unique or different genotype not in common with other isolates within the population are considered to have resulted from reactivation of latent infection, presumably acquired either outside of the population or at the time or period of interest. The assumption is that the rate of change of the molecular marker used to determine the genotype is rapid enough to show variation in a local community but slow enough that it is unlikely to change within a person in a shorter time period. If a patient has recurrent TB (*i.e.* more than one episode of active TB) and the isolates from the initial and the recurrent case are available, it is also possible to differentiate between relapse (*i.e.* TB caused by the same strain that caused the previous episode) and re-infection (*i.e.* TB caused by a different strain) (Foxman and Riley 2001).

Among the different molecular techniques, IS6110 restriction fragment length polymorphism is considered as the gold standard for molecular epidemiology of *M. tuberculosis* strains, but the method requires subculturing and DNA isolation with slow turnaround time and laborious process (Mathema *et al*. 2006). Spoligotyping, a rapid and highly reproducible genotyping tool, was developed to provide information on the structure of the DR region in the genome of individual *M. tuberculosis* strains and in different members of the *M. tuberculosis* complex (MTBC) (Kamerbeek *et al*. 1997). The simplicity of this method has facilitated the establishment of an international spoligotype database, which describes 39,295 entries from 122 countries and serves as a valuable reference for TB researchers (Brudey *et al*. 2006). Alignment of the spoligotype patterns has allowed scientists and researchers to group isolates according to similarity and to create clades or strain families. In addition, certain distinctive spoligotype patterns have been linked to defined species of the MTBC (Filliol *et al*. 2002). Another genotyping method is the MIRU-VNTR typing, a PCR (polymerase

chain reaction)-based typing method that determines the size and repeated number of units in each locus by amplifying mycobacterial interspersed repetitive units. Easy operation, economical cost, reproducible results, and high discriminatory power make it practical for routine use, and the digital results from this method can be compared and exchanged easily between different laboratories (Mears *et al*. 2015). MIRU-VNTR typing initially used 12 loci for *M. tuberculosis* strain genotyping with better resolution than Spoligotyping (Mathema *et al*. 2006). Subsequently, a new MIRU-VNTR format – which uses 15 loci (6 from the previous 12-loci version and 9 new ones) – has been shown to be more efficient at assigning clusters confirmed by epidemiological data and, therefore, can be used as a tool for real-time genotyping (Supply *et al*. 2006). In this study, Spoligotyping and 15-loci MIRU VNTR typing are used to describe the *M. tuberculosis* isolates obtained from TB cases identified in prisons.

The present study aims to describe the molecular and genotypic characteristics of *M. tuberculosis* isolates using Spoligotyping and 15-loci MIRU-VNTR typing among inmates with PTB in selected prisons in the Philippines, identify genotype clustering of TB cases, and describe a possible association of *M. tuberculosis* sublineage identified with specific demographic characteristics of the host, as well as molecular and microbiologic characteristics of the organism. To date, no study has yet been done to characterize the *M. tuberculosis* strains in the prison setting in the Philippines utilizing molecular epidemiologic and genotyping techniques.

MATERIALS AND METHODS

Sample Population

The *M. tuberculosis* isolates were obtained from a cross-sectional study conducted by Borja *et al.* (2011) in seven prisons: Cebu City Jail, Manila City Jail, Metro Manila District Jail, Antipolo City Jail, Davao City Jail, National Bilibid Prison (NBP), and Correctional Institute for Women. These prisons were selected by the Task Force for TB in Prisons Prevention and Control Program on the basis of their readiness to implement the program. The study commenced in early-2009 and ended in 2010. The study aimed to describe the prevalence of TB in selected prison sites.

Inclusion criteria were: 1) individuals already incarcerated and jail officers already appointed at the start of the study in the seven selected jails and prisons; 2) at least 18 yr of age; and 3) with signed informed consent. Those who were unable to follow study procedures and those who refused to sign an informed consent were excluded from the study.

Data Collection

All randomly selected inmates and jail officers in the various prisons underwent a general orientation on the purpose and procedures for the study. A one-on-one informed consent was taken per inmate and jail guard. All inmates and jail officers sampled for the study were screened for TB using both chest radiography and TB symptom questionnaire. All those who had a cough for 2 wk or more and/or had chest X-ray lesions suggestive of TB were requested to submit two sputum specimens for bacteriologic evaluation – namely, direct sputum smear microscopy and TB culture. The chest x-ray films were read independently by two senior radiology consultants, and an umpire reader was consulted in case of discrepancies. Sputum specimens were transported to Philippine Tuberculosis Society, Inc. Central Laboratory within 24–72 h after collection for acid-fast bacilli (AFB) staining, culture, and drug susceptibility testing (DST).

Data collected included socio-demographic data, underlying co-morbidities, and specific symptoms – including cough of two weeks or longer duration, sputum production, weight loss in the last 3 mo, recent loss of appetite, and chest pain. Other symptoms suggestive of TB were also asked, such as the presence of prolonged fever, night sweats, back pain, and hemoptysis. Any history of intake of anti-TB drugs was likewise obtained.

A total of 2,622 subjects were invited to be part of the study but only 2,450 gave their informed consent to participate. These 2,450 subjects then completed the survey questionnaire, and 1,204 were identified as TB suspects based on a set of criteria consisting of presence of symptoms (cough of at least 2-wk duration, whether continuous or intermittent, with or without other non-specific symptoms of fever, night sweats, and weight loss) and/or chest x-ray results consistent with TB – which may include a) upper lobe infiltrates (apical or posterior segments of the upper lobe) appearing as an ill-defined alveolar filling process, fibronodular pattern, or both; b) cavities; c) lymphadenopathies; and d) presence of calcification. However, only 1,120 submitted sputum for culture. Out of this, 42 had positive sputum cultures for TB. Isolates were stored at the Quezon Institute Microbiology Laboratory, and only 25 were retrieved and successfully revived for purposes of this study (Figure 1).

M. tuberculosis DNA Extraction and Genotyping

Genomic DNA was extracted from the *M. tuberculosis* isolates by resuspending mycobacterial colonies in 100–200 mL of distilled water, followed by incubation at 85 ºC for 30 min. After the suspension was centrifuged, the supernatant containing the DNA was removed and stored at –20 ºC until used for analysis.

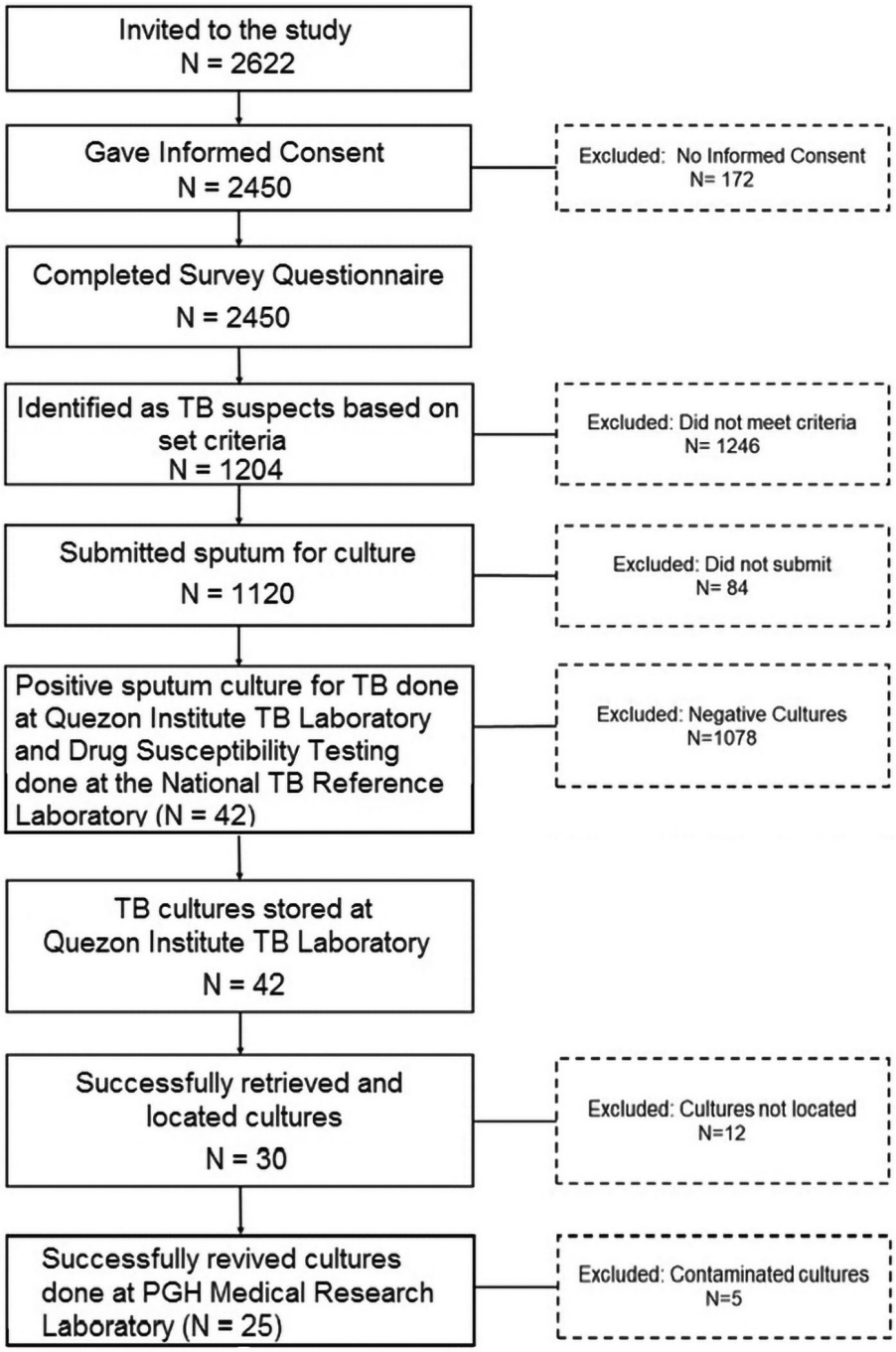


Figure 1. Patient recruitment and flow chart.

Spoligotyping was performed on all of the isolates according to the standardized protocol of Kamerbeek *et al.* (1997). Family name and SIT number (n^{o}) was assigned based on SpolDB4 (up to SIT1939) (Brudey *et al.* 2006). MIRU-VNTR typing was also performed, as in previous studies (Montoya *et al*. 2013), on all of the isolates using agarose gel electrophoresis based on a subset of 15 loci (MIRU 4, 10, 16, 26, 31, 40; Mtub 04, 21, 30, 39; ETR A, C; and QUB-11b, -26, -4156) (Supply *et al.* 2006).

Data Analysis

Descriptive statistics of the patient's socio-demographic characteristics and genetic outcomes, identified genotype families based on Spoligotyping and MIRU-VNTR typing using 15 loci were described. Proportions and associations between the patient characteristics (*e.g.* age, sex, bacillary load based on smear positivity, as well as the severity of PTB among different genotype families) were determined using Pearson's chi-squared test. The

extents of association were expressed as an odds ratio with a 95% confidence interval. Univariate and logistic regression analyses were done for possible predictors of clade or SIT. All statistical tests were two-sided and statistical significance was set at a p-value < 0.05.

M. tuberculosis strains in this study were classified into groups based on clustering. A cluster is defined as a group of two or more strains with identical genetic patterns defined either by the VNTR typing or by Spoligotyping, and strains with unmatched or unique genetic characteristics were considered non-clustered. Based on previous literature, the presence of clustering or clusters may suggest recent transmission of *M. tuberculosis*, and the clustering rate may be used to quantify recent transmission in the population. The strains with the same genetic pattern may represent an epidemiologically linked cluster.

The clustering rate is defined as $(N_c - n_c) / N_o$, where N_o is the total number of cases in the sample, n_c is the number of clusters, and N_c is the total number of cases in clusters of two or more patients. A unique strain is a genotype that is not identical to any other genotypes by a specific molecular typing method. The recent transmission index was computed using the (n – 1) method in which the number of isolates in clusters minus the number of clusters divided by the total number of isolates represents the recent transmission rate.

The HGDI equation was also calculated for each method (Spoligotyping and MIRU-VNTR typing) and combined using the formula:

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

where D is the index of discriminatory power, a_j is the number of strains in the population that are indistinguishable from the jth strain, and N is the number of strains in the population. D can take any figure between 0–1 while the lowest and largest discriminatory power indexes are represented by 0 and 1, respectively.

Ethical Considerations

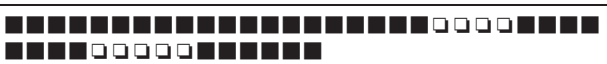
Potential study participants were informed of the nature and rationale of the study using an information sheet in Filipino. Separate written informed consent on study participation and specimen banking were also obtained. The study protocol was approved by the Institutional Ethics Committee of the Quezon Institute, Quezon City, Philippines.

RESULTS

A total of 25 *M. tuberculosis* isolates from 25 individual inmates in selected prisons were collected from the study by Borja *et al.* (2011). All of these isolates underwent Spoligotyping and 15-loci MIRU-VNTR typing. Table 1 shows the distribution of lineage and SITs of the isolates used in this study.

Table 1. Distribution of lineage and SIT of the isolates in the study.

SIT[a]	Sublineage[b]	Spoligotype octal	Spoligotype binary	Number of isolates
19	EAI2_Manila	677777477413771	■■❑■■■■■■■■■■■■■■■■❑❑■■■■■■■ ❑❑❑❑■❑■■■■■■■■■	10
17	LAM2	677737607760771	■■❑■■■■■■■■■❑■■■■■■■❑❑❑❑■■■■ ■■■■❑❑❑❑■■■■■■■	4
2074	EAI2_Manila	677777457413771	■■❑■■■■■■■■■■■■■■■■❑❑■❑■■■■■■ ❑❑❑❑■❑■■■■■■■■■	1
Orphan	EAI2_Manila	667777477413771	■■❑■■❑■■■■■■■■■■■■■■❑❑■■■■■■■ ❑❑❑❑■❑■■■■■■■■■	1
1	Beijing	000000000003771	❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑❑ ❑❑❑❑❑❑■■■■■■■■■	1
42	LAM9	777777607760771	■■■■■■■■■■■■■■■■■■❑❑❑❑■■■■ ■■■■❑❑❑❑■■■■■■■	1
64	LAM6	777777607560771	■■■■■■■■■■■■■■■■■■❑❑❑❑■■■■ ❑■■■❑❑❑❑■■■■■■■	1
396	LAM6	777777607560371	■■■■■■■■■■■■■■■■■■❑❑❑❑■■■■ ❑■■■❑❑❑❑❑■■■■■■	1

SIT[a]	Sublineage[b]	Spoligotype octal	Spoligotype binary	Number of isolates
1070	LAM9	777777607760371		1
1694	LAM2	277737607760771		1
NA	Unknown	676777477413771		1

[a]SIT designates spoligotypes shared by two or more patient isolates in SpolDB4;
"NA" designates spoligotypes not registered in SpolDB4.
[b]Sublineage was designated according to SpolDB4. Spoligotypes not registered in SpolDB4 were designated as "unknown" sublineage.

The majority of the patients were male (21/25 or 84%) and aged 30–49 yr (17/25 or 68%). Eighteen of 25 or 72% of the culture-positive patients had severe PTB. Thirteen (13/25) or 52% were smear-positive. Eleven (11/25) or 44% were classified as high bacillary load with a sputum smear of ≥ +3 (Table 2).

The majority of the isolates were susceptible to all the first-line drugs (18/25 or 72%). Two isolates (2/25 or 8%) were MDR (*i.e.* with resistance to both isoniazid and rifampicin). One was resistant to rifampicin, isoniazid, and streptomycin, and belonged to SIT1 with an unclassified sublineage that did not match any in the international database; the other was resistant to all of the first-line drugs (pyrazinamide was not tested) and kanamycin, and belonged to LAM6 sublineage and SIT396. Both had a history of anti-TB treatment. Both of these isolates were from patients in the same prison (NBP) but in different cells. Three other isolates (3/25 or 12%) showed monoresistance to streptomycin (Table 3).

Spoligotyping

In this study, 25 isolates were clustered into nine distinct spoligotypes. The Spoligotyping of 10 of the 25 *M. tuberculosis* isolates yielded characteristic identical patterns lacking hybridization to eight spacers (3, 20, 21, 29–32, and 34); the remaining 35 spacers showed a positive hybridization signal. This was classified as belonging to the EAI2_Manila sublineage under SIT19. Two isolates exhibited Spoligotyping patterns that were highly similar to the pattern observed for the 10 isolates. One lacked hybridization to nine spacers (3, 20, 21, 23, 29–32, and 34) and the other lacked hybridization also to nine spacers (3, 6, 20, 21, 29–32, and 34), but these two isolates were also classified under EAI2_Manila sublineage.

Table 2. Association of MTB sublineage (EAI2_Manila and other genotype strains) with age, sex, severity of pulmonary TB, and bacillary load of patients with TB in selected prisons in the Philippines.

	EAI2_Manila	Others	Total	*p*-value[c]
Age				$p = 0.546$
20–29	1	3	4	
30–39	6	3	9	
40–49	4	4	8	
50–59	1	2	3	
60–69	0	1	1	
Total	12	13	25	
Sex				$p = 0.100$
Male	10	11	21	
Female	2	2	4	
Total	12	13	25	
Severity of PTB[a]				$p = 0.378$
Non-severe	2	5	7	
Severe	10	8	18	
Total	12	13	25	
Bacillary load[b]				$p = 0.695$
Low	6	8	14	
High	6	5	11	
Total	12	13	25	

[a]Severe PTB is determined as pulmonary involvement with cavitary lesions, miliary TB, extensive involvement of one or both lungs or presence of pleural effusion.
[b]Bacillary load determined by AFB smear examination using Ziehl-Neelsen method. Low bacillary load is AFB ≤ +2 or positive only for culture. High bacillary load is AFB ≥ +3.
[c]*p*-value for Pearson's chi-squared test

Table 3. Association of MTB sublineage (EAI2_Manila and other genotype strains) with drug resistance of TB strains obtained from patients with TB in selected prisons in the Philippines.

	EAI2_Manila	Others	Total	*p*-value[a]
Drug resistance				$p = 0.590$
Susceptible	9	9	18	
Monoresistant	2	1	3	
Multidrug-resistant	0	2	2	
No data	1	1	2	
Total	12	13	25	

[a]*p*-value for Pearson's chi-squared test

The octal coded Spoligotyping results were assigned SIT numbers and then compared with those in SpolDB4 (Brudey *et al*. 2006) and the updated SITVIT (Demay *et al.* 2012) databases. Regarding the distribution of SITs, SIT 19 predominated with 10/25 (40%), followed by SIT 17 (4/25, 16%) and one each for SIT 1, 396, 64, 42, 1694, 1070, and 2074. Four strains were not identified by the international database. In total, the 25 isolates were grouped into two clusters and 13 genotypes with a clustering rate of 12.1%. There were 11 unique strains identified.

The distribution of strains according to *M. tuberculosis* sublineages in decreasing order was as follows: EAI2_ Manilla (12/25 or 48%) > LAM2 (5/25 or 20%) > LAM6 (2/25 or 8%) = LAM9 (2/25 or 8%) > Beijing (1/25 or 4%). Three strains were not registered in SpolDB4 and were designated as unknown lineage. Table 4 shows the discriminatory ability and clustering rate of the genotyping methods used. The HGDI of Spoligotyping was 0.83.

15-loci MIRU-VNTR Typing

The analysis of the *M. tuberculosis* isolates using the 15-loci MIRU-VNTR typing showed that there were 23 genotypes, 21 TB isolates of which were unique, and the remaining four isolates were grouped into two clusters. These clusters had two isolates each and had an allelic profile of 192224454324297 and 421332424243132, respectively. The clustering rate of 15-loci MIRU-VNTR typing was 3.4%. The HGDI of all the loci sets reached 0.993.

When Spoligotyping and 15-loci MIRU-VNTR typing were combined, there were still 23 genotypes, 21 of which were unique strains with four isolates grouped into two clusters with two isolates per cluster. The clustering rate was still at 3.4%. The HGDI of combined Spoligotyping and 15-loci MIRU-VNTR typing was 0.993, which was an improvement compared to using Spoligotyping alone. This means that the 15-loci MIRU-VNTR typing used in combination with Spoligotyping provides significant discriminatory power.

DISCUSSION

Mycobacterium tuberculosis is one pathogenic bacterial species in the MTBC. Seven human-adapted MTBC lineages are characterized based on the phylogenetic analysis; lineages 1–4 and 7 are *M. tuberculosis* strains, and lineages 5 and 6 are *Mycobacterium africanum*. Lineages 1, 5, and 6 are classified as ancient lineages due to the presence of a 52-bp TbD1 region, which was deleted along the evolutionary timeline (Galagan 2014). The EAI2_Manila strain belongs to Lineage 1.

Our study results show the predominance of the EAI2_ Manila sublineage in the prison setting similar to study results done in community-based settings. Our findings are consistent with a previous study that also involved Philippine *M. tuberculosis* isolates that resulted in the creation of the Manila Family or EAI2_Manila sublineage (Douglas *et al*. 2003) and a study done in a suburban community in the Philippines (Montoya *et al*. 2013), as well as more recent studies (Phelan *et al*. 2019). The EAI

Table 4. Discriminatory ability of Spoligotyping and 15-loci MIRU-VNTR typing using *M. tuberculosis* isolates from patients in selected prisons in the Philippines.

	Spoligotyping	15-loci MIRU-VNTR typing[a]	Combined Spoligotyping and 15-loci MIRU-VNTR typing
HGDI[b]	0.83	0.993	0.993
Number of clusters	2	2	2
Number of genotypes	13	23	23
Number of clustered isolates	14	4	4
Clustering rate (%)[c]	12.1	3.4	3.4
Number of unique strains	11	21	21

[a]15-loci MIRU-VNTR typing includes MIRU 4,10,16, 26, 31, 40; Mtub 04, 21, 30, 39; ETR A, C; and QUB-11b, 26, 4156.
[b]HGDI as Hunter-Gaston discrimination index
[c]Clustering rate is defined as $(N_c - n_c) / N_0$, where N_0 is the total number of cases in the sample, n_c is the number of clusters, and N_c is the total number of cases in clusters of two or more patients.

lineage has also been shown to be more prevalent in other countries in Southeast Asia like Malaysia and Vietnam (Ismail 2014). Other studies have also shown that the EAI2_Manila sublineage was also identified in other countries where large Filipino immigrant communities are located or where Filipinos constitute a significant workforce (Varghese *et al*. 2013).

Gagneux proposed an evolutionary theory to possibly explain why specific lineages predominate in certain geographic areas. More virulent TB strains may have been selected out during humanity's hunter-gatherer period. Low population densities meant that more virulent strains would quickly decimate their susceptible hosts and therefore disappear, while less virulent strains may persist in hosts after a period of latency. As the human population increased, more virulent strains would have seen higher transmission and greater spread but with increased mortality. Thus, Gagneux proposed that the ancient lineage 1 (including the Manila family) evolved towards lower virulence, while the modern lineages evolved with increased virulence. This may partially explain the Manila family's predominance in the Philippines with limited transmission outside of the country (Gagneux 2013). This may also have something to do with the increased transmissibility of this strain in the country. Differences in transmissibility of *M. tuberculosis* strains from patients to contacts have been shown based on specific TB strains. For example, lineages 2 and 4 are often considered as the most transmissible strains, which could reflect increased virulence. It was also observed that lineage 1 strains – where EAI2_Manila belongs – were associated with over-transmission in East Asia only, which suggests that geographic location may have some effect on the behavior of specific strains (Wiens *et al.* 2018).

A possible association between patient demographic factors and the EAI2_Manila sublineage was also analyzed. No significant association was shown between EAI2_Manila sublineage and sex and age groups. No significant association was also seen between EAI2_Manila sublineage and severity of PTB, bacillary load, and resistance to anti-TB drugs. This lack of associations was also consistent with a previous community-based study looking at the association of EAI2_Manila clade with host demographic factors and disease characteristics (Montoya *et al*. 2013). However, there is mounting experimental evidence to suggest that *M. tuberculosis* lineages interact differently with the immune system of the human host, potentially leading to distinct clinical manifestations (Tientcheu *et al*. 2017).

There is limited evidence to show that variable *M. tuberculosis* lineages may have variable human host presentations and outcomes. Some human studies have shown that high virulence *M. tuberculosis* strains associated with lineage 2 were associated with different levels of cytokine and immune response and different rates of bacterial multiplication (Tram *et al*. 2018). For example, in a study involving Vietnamese TB patients using a monocyte-derived macrophage lysis model, a high degree of macrophage lysis (*i.e.* high virulence) was associated with higher bacterial concentrations in sputum. In our study, we hoped to see this association using sputum AFB smear grading with TB sublineage, but the small sample size did not make this possible. In the same study, high virulence strains also replicated faster in the macrophages and induced lower secretion of tumor necrosis factor α (TNF-α) and IL-6 but higher production of IL-1β (Tram *et al*. 2018). These may have implications on the severity of disease and clinical outcomes.

Interaction of host ethnicity and *M. tuberculosis* lineage may also have differential outcomes. In a study looking at this possible interaction, both Filipino ethnicity and *M. tuberculosis* strain CDC1551 were shown to be significant predictors of cytokine production (Nahid *et al*. 2018). The sample size, however, was small and live bacterial challenges may have to be done.

There are also other studies that have shown specific strains of *M. tuberculosis* to be associated with hypervirulence leading to severe forms of TB. The mechanism behind this is the ability of the organism to multiply inside the macrophage and evade immune mechanisms, favoring its dissemination to other organs and causing more severe forms of TB disease. However, there are also other studies that show a lack of association. In one study, the EAI lineage was associated with lower rates of TB transmission, as measured by positive tuberculin skin test among close contacts of pulmonary cases. There was also no increased risk of disease severity. On the other hand, the Beijing strain was associated with greater virulence and transmissibility of infection and severity (Albanna *et al.* 2011; Konstantynovska *et al*. 2019). There is, therefore, a need to do more studies to find out associations between EAI2_Manila sublineage and severity of the disease.

Interestingly, next to EAI2_Manila sublineage, we have seen the emergence of the LAM lineage broken down into LAM2 (five isolates), LAM6 (two isolates), and LAM9 (two isolates) comprising a total of nine or 36% of the total isolates. The LAM lineage has been reported in some studies to be associated with patients who have incarceration histories. Studies done in prisons in Southern Brazil and Tula Region, Russia also showed that this family is widespread. DST also revealed a high rate of MDR isolates in this group (Ignatova *et al.* 2006; Medeiros *et al*. 2018).

The clustering rate of 3.4% in this study is low compared to the clustering rate described in similar studies in the

prison setting in other countries (Huber *et al.* 2014). The relatively low clustering rate may indicate a significant level of genotypic diversity of the *M. tuberculosis* isolates that may be more suggestive of reactivation of a past or latent infection rather than a recent active transmission that may indicate an outbreak.

The combination of Spoligotyping and MIRU-VNTR typing, where patterns must match in both methods to be considered a transmission link, is often considered the molecular gold standard for transmission linking and genotyping (Supply *et al*. 2006). Although the determination of clustering rates using the n-1 technique may indicate local transmission, there may also be an overestimation. Reports comparing MIRU-VNTR typing to whole-genome sequencing (WGS) have shown that genotype-level identity does not always correspond to genomic distances that reflect recent local transmission. In addition to defining temporal, physical, and spatial epidemiologic links of cases identified, WGS and phylogenetic analysis using a much larger (90%) portion of the *M. tuberculosis* genome may have to be done to prove ongoing transmission (Jamieson *et al*. 2014). Patient-level factors or coexisting diseases, such as HIV infection and drug or alcohol use, may also be associated with increased odds of clustering, consistent with other studies (Mehaffy *et al.* 2014). This information, however, was not available to researchers in this study.

Several studies indicate a strong association between drug resistance and specific *M. tuberculosis* strains such as the Beijing strain (Galagan 2014). We were able to identify only two MDR isolates in our study; hence, definite conclusions on the association between drug resistance and EAI2_Manila sublineage cannot be made.

It will be difficult to make definitive conclusions on the epidemiology of TB in prisons in the Philippines because of the few *M. tuberculosis* isolates obtained from active cases, but this gives us an initial genotypic description of the isolates obtained from cases in selected prisons in the Philippines. More studies have to be done on more prison sites utilizing more advanced and sensitive genotyping techniques, such as WGS, to better describe the dynamics of TB transmission in Philippine prisons.

CONCLUSION

The predominant sublineage of *M. tuberculosis* infecting TB patients in selected prisons in the Philippines is the EAI2_Manila sublineage, and the predominant SIT is SIT 19 by Spoligotyping. This is consistent with previously published studies on the common clades of *M. tuberculosis* in the Philippines. Most of the TB patients affected were relatively young (30–49 yr of age). No association was seen between EAI2_Manila clade and sex and age of patients. There was also no significant association seen between the EAI2_Manila sublineage and sputum smear positivity and severity of PTB.

ACKNOWLEDGMENTS

The authors extend their gratitude to the National Research Council of the Philippines (under the Department of Science and Technology Philippines), the Research Institute of Tuberculosis Japan, and the Japan Anti-Tuberculosis Association for supporting this study.

STATEMENT ON CONFLICT OF INTEREST

No conflict of interest was declared.

REFERENCES

ALBANNA A, REED M, KOTAR K, FALLOW A, MCINTOSH F, BEHR M, MENZIES D. 2011. Reduced Transmissibility of East African Indian Strains of *Mycobacterium tuberculosis*. PLoS One 6(9): e25075. doi: 10.1371/journal.pone.0025075

BORJA M, MORTERA L, PALASI W, BENEDICTO J, CADENA E. 2011. Prevalence of bacteriologically confirmed pulmonary tuberculosis among prisoners and jail officers in selected prisons in the Philippines. Retrieved on 06 Jun 2013 from http://www.herdin.ph/index.php?view=research&cid=39266

BRUDEY K, DRISCOLL J, RIGOUTS L, PRODINGER W, GORI A, AL HAJOJ S, ALLIX C, ARISTIMUNO L, ARORA J, BAUMANIS V, BINDER L, CAFRUNE P, CATALDI A, CHEONG S, DIEL R, ELLERMEIER C, EVANS J, FAUVILLEDUFAUX M, FERDINAND S, GARCIA DE VIEDMA D, GARZELLI C, GAZZOLA L, GOMES H, GUTIERREZ M, HAWKEY P, VAN HELDEN P, KADIVAL G, KREISWIRTH B, KREMER K, KUBIN M, KULKARNI M, LIENS B, LILLEBAEK T, HO M, MARTIN C, MOKROUSOV I, NARVSKAIA O, NGEOW F, NAUMANN L, NIEMANN S, PARWATI I, RAHIM Z, RASOLOFORAZANAMPARANY V, RASOLONAVALONA T, ROSSETTI M, RUSCH-GERDES S, SAJDUDA A, SAMPER S, SHEMYAKIN I, SINGH U, SOMOSKOVI A, SKUCE R, VAN SOOLINGEN D, STREICHER E, SUFFYS P,

TORTOLI E, TRACEVSKA T, VINCENT V, VICTOR T, WARREN R, YAP S, ZAMAN K, PORTAELS F, RASTOGI N, SOLA C. 2006. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international Spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiol 6: 23. doi: 10.1186/1471-2180-6-23

DEMAY C, LIENS B, BURGUIÈRE T, HILL V, COUVIN D, MILLET J, MOKROUSOV I, SOLA C, ZOZIO T, RASTOGI N. 2012. SITVITWEB - a publicly available international multimarker database for studying *Mycobacterium tuberculosis* genetic diversity and molecular epidemiology. 2012. Infect Genet Evol 12(4): 755–766. doi: 10.1016/j.meegid.2012.02.004

[DOH] Department of Health. 2017. National Tuberculosis Prevalence Survey 2016, Philippines. Retrieved on 01 Dec 2017 from http://ntp.doh.gov.ph/downloads/publications/NTPS2016.pdf

DOUGLAS J, QIAN L, MONTOYA J, MUSSER J, VAN EMBDEN J, VAN SOOLINGEN D, KREMER K. 2003. Characterization of the Manila Family of *Mycobacterium tuberculosis*. J Clin Microbiol. 41(6): 2723–2726. doi: 10.1128/JCM.41.6.2723-2726.2003

FILLIOL I, DRISCOLL J, VAN SOOLINGEN D, KREISWIRTH B, KREMER K, VALETUDIE G, ANH D, BARLOW R, BANERJEE D, BIFANI P, BRUDEY K, CATALDI A, COOKSEY R, COUSINS D, DALE J, DELLAGOSTIN A, DROBNIEWSKI F, ENGELMANN G, FERDINAND S, GASCOYNE-BINZI D, GORDON M, GUTIERREZ M, HAAS W, HEERSMA H, KALLENIUS G, KASSA-KELEMBHO E, KOIVULA T, LY H, MAKRISTATHIS A, MAMMINA C, MARTIN G, MOSTROM P, MOKROUSOV I, NARBONNE V, NARVSKAYA O, NASTASI A, NIOBE-EYANGOH S, PAPE J, RASOLOFO-RAZANAMPARANY V, RIDELL M, ROSSETTI M, STAUFFER F, SUFFYS P, TAKIFF H, TEXIER-MAUGEIN J, VINCENT V, DE WAARD J, SOLA C, RASTOGI N. 2002. Global distribution of *Mycobacterium tuberculosis* spoligotypes. Emerg Infect Dis 8: 1347–1349.

FOXMAN B, RILEY L. 2001. Molecular epidemiology: focus on infection. Am J Epidemiol 153(12): 1135–1141.

GALAGAN JE. 2014. Genomic insights into tuberculosis. Nat Rev Genet 15: 307–320. https://doi.org/10. 1038/nrg3664 PMID: 24662221

GAGNEUX S. 2013. Genetic Diversity in *Mycobacterium tuberculosis*. Current Topics in Microbiology and Immunology 374: 1–25. doi: 10.1007/82_2013_329

HUBER F, SANCHEZ A, GOMES H, VASCONCELLOS S, MASSARI V, BARRETO A, CESCONI V, MACHADO S, GOMGNIMBOU M, SOLA C, LAROUZE B, SUFFYS P, SAAD M. 2014. Insights into the population structure of *Mycobacterium tuberculosis* using Spoligotyping and RDRio in a southeastern Brazilian prison unit. Infect Genet Evol 26: 194–202. doi: 10.1016/j.meegid.2014.05.031

IGNATOVA A, DUBILEY S, STEPANSHINA V, SHEMYAKIN I. 2006. Predominance of multi-drug resistant LAM and Beijing family strains among *Mycobacterium tuberculosis* isolates recovered from prison inmates in Tula Region, Russia. J Med Microbiol 55: 1413–1418. doi: 10.1099/jmm.0.46575-0

ISMAIL F, COUVIN D, FARAKHIN I, RAHMAN Z, RASTOGI N, SURAIYA S. 2014. Study of *Mycobacterium tuberculosis* Complex Genotypic Diversity in Malaysia Reveals a Predominance of Ancestral East-African-Indian Lineage with a Malaysia-Specific Signature. PLoS ONE 9(12): e114832. doi: 10.1371/journal.pone.0114832

IJAZ K, YANG Z, TEMPLETON G, STEAD WW, BATES JH, CAVE MD. 2004. Persistence of a strain of *Mycobacterium tuberculosis* in a prison system. Int J Tuberc Lung Dis 8: 994–1000. PMID: 15305483

JAMIESON FB, TEATERO S, GUTHRIE JL, NEEMUCHWALA A, FITTIPALDI N, MEHAFFY C. 2014. Whole-genome sequencing of the *Mycobacterium tuberculosis* Manila sublineage results in less clustering and better resolution than mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) typing and Spoligotyping. J Clin Microbiol 52: 3795–3798. doi: 10.1128/JCM.01726-14

KAMERBEEK J, SCHOULS L, KOLK A, VAN AGTERVELD M, VAN SOOLINGEN D, KUIJPER S, BUNSCHOTEN A, MOLHUIZEN H, SHAW R, GOYAL M, VAN EMBDEN J. 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 35(4): 907–914. PMID 9157152.

KONSTANTYNOVSKA O, REKROTCHUK M, HREK I, ROHOZHYN A, RUDOVA N, POTEIKO N, GERILOVYCH A, BORTZ E, SOLODIANKIN O. 2019. Severe Clinical Outcomes of Tuberculosis in Kharkiv Region, Ukraine, Are Associated with Beijing Strains of *Mycobacterium tuberculosis*. Pathogens 8: 75. doi: 10.3390/pathogens 8020075

MATHEMA B, KUREPINA NE, BIFANI PJ, KREISWIRTH BN. 2006. Molecular Epidemiology of Tuberculosis: Current Insights. Clinical Microbiology Reviews 19(4): 658–685.

MEARS J, ABUBAKAR I, COHEN T, MCHUGH TD, SONNENBERG P. 2015. Effect of study design and setting on tuberculosis clustering estimates using mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR): a systematic review. BMJ Open 5(1): e005636.

MEDEIROS T, NOGUEIRA C, PRIM R, SCHEFFER M, ALVES E, ROVARIS D, ZOZIO T, RASTOGI N, BAZZO M. 2018. Molecular epidemiology of *Mycobacterium tuberculosis* strains from prison populations in Santa Catarina, Southern Brazil. Infect Genet Evol 58: 34–39. doi: 10.1016/j.meegid.2017.12.010

MEHAFFY C, GUTHRIE JL, ALEXANDER DC, STUART R, REA E, JAMIESON FB. 2014. Marked microevolution of a unique *Mycobacterium tuberculosis* strain in 17 years of on-going transmission in a high risk population. PLoS One 9: e112928.

MONTOYA J, MURASE Y, ANG C, SOLON J, OHKADO A. 2013. A Molecular Epidemiologic Analysis of *Mycobacterium tuberculosis* among Filipino Patients in a Suburban Community in the Philippines. Kekkaku [Tuberculosis] 88(6): 543–552.

NAHID P, JARLSBERG L, KATO-MAEDA M, SEGAL M, OSMOND D, GAGNEUX S, DOBOS K, GOLD M, HOPEWELL P, LEWINSOHN D. 2018. Interplay of strain and race/ethnicity in the innate immune response to *M. tuberculosis*. PLoS One 13(5): e0195392. doi: 10.1371/ journal.pone.0195392

PHELAN J, LIM D, MITARAI S, DE SESSIONS P, TUJAN MA, REYES L, MEDADO I, PALPARAN A, NAIM A, JIE S, SEGUBRE-MERCADO E, SIMOES B, CAMPINO S, HAFALLA J, MURASE Y, MORISHIGE Y, HIBBERD M, KATO S, AMA MC, CLARK T. 2019. *Mycobacterium tuberculosis* whole genome sequencing provides insights into the Manila strain and drug-resistance mutations in the Philippines. Nature Scientific Reports 9: 9305. https://doi.org/10.1038/s41598-019-45566-5

SUPPLY P, ALLIX C, LESJEAN S, CARDOSO-OELEMANN M, RUSCH-GERDES S, WILLERY E, SAVINE E, DE HAAS P, VAN DEUTEKOM H, RORING S, BIFANI P, KUREPINA N, KREISWIRTH B, SOLA C, RASTOGI N, VATIN V, GUTIERREZ MC, FAUVILLE M, NIEMANN S, SKUCE R, KREMER K, LOCHT C, VAN SOOLINGEN D. 2006. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. J Clin Microbiol 44(12): 4498–4510. doi: 10.1128/JCM.01392-06

TIENTCHEU L, KOCH A, NDENGANE M, ANDOSEH G, KAMPMANN B, WILKINSON R. 2017. Immunological consequences of strain variation within the *Mycobacterium tuberculosis* complex. Eur J Immunol 47(3): 432–445. doi: 10.1002/eji.201646562

TRAM T, NHUNG H, VIJAY S, HAI H, THU D, HA V, DINH T, ASHTON P, HANH N, PHU N, THWAITES G, THUONG N. 2018. Virulence of *Mycobacterium tuberculosis* clinical isolates is associated with sputum pre-treatment bacterial load, lineage, survival in macrophages, and cytokine response. Front Cell Infect Microbiol 8: 417. doi: 10.3389/ fcimb.2018.00417

VARGHESE B, SUPPLY P, SHOUKRI M, ALLIX-BEGUEC C, MEMISH Z, ABULJADAYEL N, AL-HAKEEM R, ALRABIAH F, AL-HAJOJ S. 2013. Tuberculosis Transmission among Immigrants and Autochthonous Populations of the Eastern Province of Saudi Arabia. PLoS ONE 8(10): e77635. doi: 10.1371/journal.pone.0077635

WIENS K, WOYCZYNSKI L, LEDESMA J, ROSS J, ZENTENO-CUEVAS R, GOODRIDGE A, ULLAH I, MATHEMA B, SIAWAYA J, BIEHL M, RAY S, BHATTACHARJEE N, HENRY N, REINER C JR, KYU H, MURRAY C, HAY S. 2018. Global variation in bacterial strains that cause tuberculosis disease: a systematic review and meta-analysis. BMC Med 16(1): 196. doi: 10.1186/s12916-018-1180-x

[WHO] World Health Organization. 2019. Global Tuberculosis Report 2019. Retrieved on 01 Jan 2020 from https://www.who.int/tb/publications/global_report/en/