A MOLECULAR EPIDEMIOLOGIC ANALYSIS of *Mycobacterium tuberculosis* among FILIPINO PATIENTS in a SUBURBAN COMMUNITY in the PHILIPPINES

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Abstract

Background: The Philippines is designated as one of the high tuberculosis (TB) burden countries by WHO. We conducted a molecular epidemiologic analysis of *Mycobacterium tuberculosis* isolates collected from patients consulting at the health clinics in the city of Santa Rosa, Laguna, a suburban community in the Philippines.

Methods: A total of 116 *M. tuberculosis* isolates were characterized and genotyped using spoligotyping and 15 loci of variable number of tandem repeats of mycobacterial interspersed repetitive units (15 MIRU-VNTR). The strains were then compared with the international spoligotyping database (SpolDB4). Cluster analyses were done using 15 MIRU-VNTR and spoligotyping.

Results: Majority of the patients with pulmonary tuberculosis were young (18-29 year age group at 41.4%) and male (62.1%). 86 / 116 (74.1%) were sputum-smear positive and 43 / 116 (37.1%) had severe pulmonary tuberculosis. When the genotyping results were compared to the SpoIDB4, there were 14 identified Spoligo-International-Types (SITs) with SIT19 as the predominant SIT (81 / 116, 69.8%). 14 out of 116 (12.1%) did not match any SIT in the SpoIDB4. The distribution of strains according to major *M. tuberculosis* clades was as follows: EAI2_Manilla (96 / 116, 82.8%; U 3 / 116, 2.6%; LAM2 1 / 116, 0.9%; EAI3_IND 1 / 116, 0.9%; MANU2 1 / 116, 0.9%. Using univariate and multivariate analysis, there was no significant association shown between the

EAI2_Manilla clade and SIT with patient characteristics such as sex and age groups as well as bacillary load based on sputum-smear positivity and severity of pulmonary tuberculosis. Using logistic regression, no patient characteristic, as well as bacillary load or severity of TB, were significant predictors for clade or SIT. Based on the molecular typing method used, spoligotyping identified 5 clusters and 27 genotypes (22 unique strains) with a Hunter Gaston Discrimination Index (HGDI) of 0.511. 15 MIRU-VNTR identified 16 clusters and 69 genotypes (53 unique strains) with an HGDI of 0.975. The combination of spoligotyping and 15 MIRU-VNTR identified 10 clusters and 83 genotypes (73 unique strains) with the highest HGDI at 0.975. High case rate of TB among young people in this community suggests the high transmission rate of infection. However, in the absence of significant association between clustering and age, the interpretation of observed high cluster rate warrants caution, and requires further molecular and epidemiological observation.

Conclusion: This is the first molecular epidemiology study to show the distribution of genotypes of the *M. tuberculosis* strains, systematically and prospectively sampled, of the patient population in a suburban community in the Philippines. The combination of spoligotyping and 15 MIRU-VNTR identified 10 clusters and 83 genotypes (73 unique strains) with the highest HGDI at 0.975. High case rate of TB among young people in this community suggests the high transmission of infection. However, in the absence of significant association between clustering and age, the interpretation of

observed high cluster rate warrants caution, and requires further molecular and epidemiological observation. .

BACKGROUND

Tuberculosis (TB), long known to be a major cause of morbidity and mortality throughout the world, has for the past several decades been a neglected disease in both industrialized and developing countries. In 2011, there were an estimated 8.7 million new cases of TB (13% co-infected with HIV) and 1.4 million deaths from TB[1)]. Most of the cases were in developing countries where *Mycobacterium tuberculosis* (*M. tuberculosis*) transmission has been associated with factors like crowding and poor or weak public health infrastructure[2)]. In the Philippines, tuberculosis is the fifth leading cause of morbidity and mortality in the general population[3)]. It is one of the twenty-two high burden countries that account for 80% of the world's TB cases. Approximately 150,000 new smear-positive cases of pulmonary TB are identified every year which represents one-third of the total TB cases, majority of which are smear-negative. This heavy burden of TB may further be compounded by the problems of HIV and multi-drug resistant TB in the country.

One of the identified strategies for TB control is the conduct of molecular epidemiologic studies that will describe transmission patterns of TB and characterization of the circulating *M. tuberculosis* strains. Recent advances in molecular microbiology have allowed the development of molecular tools for the genetic analysis of *M. tuberculosis* strains, which subsequently can provide better insights on the epidemiology of TB. This molecular epidemiology approach, that combines molecular biology with epidemiology, statistics and clinical medicine, permits the formulation of

more effective and targeted control strategies. These studies can estimate the fraction of cases attributable to recent transmission or reactivation, confirm laboratory based errors, distinguish endogenous reactivation and exogenous reinfection and identify routes of transmission of infection. Also, it is useful for investigating patterns of drug resistance with specific populations or groups of strains to better understand transmission dynamics within specific populations[4]. Molecular genotyping tools for tuberculosis include several technologies such as IS*6110*-based restriction fragment length polymorphism (RFLP), spoligotyping, 15 loci of variable number of tandem repeats of mycobacterial interspersed repetitive units (15 MIRU-VNTR) and single nucleotide polymorphism (SNP).

The Philippines has very limited data regarding the molecular epidemiology of *M. tuberculosis* isolates in the country. In the initial study done by Douglas *et al*, the isolates of *M. tuberculosis* from Filipino patients without HIV infection were found to belong to a distinct family of TB strains, which may be called the Manila family of *M. tuberculosis*, different from the identified strains in the Asian region based on RFLP and spoligotyping analysis[5]. The Manila family of *M. tuberculosis* has also been described among Filipino patients with TB in countries like the United States where large immigrant Filipino communities are located[5].

The present study aims to characterize the strains of *M. tuberculosis* in adult Filipino patients in the city of Santa Rosa, a suburban community in the Philippines through molecular analytic methods, identify genotype clustering of TB cases that may indicate active TB transmission, and to

describe possible association of transmission with specific demographic characteristics of the host as well as molecular and microbiologic characteristics of the organism.

MATERIALS AND METHODS

Study Population and Mycobacterial strains

Sputum specimens were collected consecutively from all adult patients (age 18-64 years old) who consulted at the City Health Office and the Barangay Health Stations of the city of Santa Rosa , Laguna, Philippines for evaluation and management of possible pulmonary TB from March 2009 to June 2010, and who were assessed to be eligible for inclusion in the study. The study excluded patients already on treatment for TB for more than 7 days or a history of previous tuberculosis treatment as these may lead to negative culture results. Patients with extrapulmonary TB were also excluded.

Three sputum samples were sent to the Tuberculosis Laboratory, Medical Research Laboratories, Philippine General Hospital (PGH). Acid-fast bacilli smear examination on concentrated sputum was done as well as culture for *M. tuberculosis* using Loewenstein-Jensen culture medium. For the purpose of this study, a chest radiography was also obtained.

Genomic DNA extraction

Genomic DNA was extracted from the *M. tuberculosis* isolates[6]. The mycobacterial colonies were re-suspended in 100 to 200 ul of distilled water and boiled at 100^0C for 15 minutes to obtain genomic DNA. After the suspension was centrifuged, the supernatant containing the DNA was removed and stored at -20^0C until used for analysis.

Genotyping

Spoligotyping was performed on all of the isolates according to the standardized protocol of Kamerbeek *et al*[7]. Family name and SIT number (Spoligo-International Type number) was assigned based on SpolDB4 (up to SIT1939)[8]. 15 MIRU-VNTR typing was performed as previously described[9] on all of the isolates using agarose gel electrophoresis based on a subset of 15 loci, which was proposed as the international standard for routine epidemiological discrimination of *M. tuberculosis* strains[10]. The subset includes MIRU 4, 10, 16, 26, 31, 40; Mtub 04, 21, 30, 39; ETR A, C; and QUB-11b, -26, -4156[10].

Data Analysis

Frequencies of identified genotype families based on spoligotyping and 15 MIRU-VNTR using 15 loci were described. Frequencies of patient characteristics and smear positivity as well as severity of pulmonary TB among different genotype families were compared using Pearson chi-square test. The extent of association was expressed as an odds ratio (OR) with a 95% confidence interval (95%C.I.). Univariate and multivariate analysis were done with logistic regression for

possible predictors of clade or SIT. All statistical tests were two-sided and statistical significance was set at a p value of <0.05. The 15 MIRU-VNTR dendrogram was built with the unweighted pair group method for mathematical averages (UPGMA), using BioNumerics® (v.5.1 Applied Maths, Sint Martin Latems, Belgium).

TB strains in this study can be classified into two groups, clustered or non-clustered *M. tuberculosis* isolates. A cluster is defined as a group of two or more strains with identical genetic patterns defined by 15 MIRU-VNTR typing and/or by spoligotyping and strains with unmatched or unique genetic characteristics were considered non-clustered. Clustering rate corrected using "n-1 method" 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[11]. This is assumed to represent the recent transmission rate. Hunter-Gaston Discrimination index (HGDI) was computed in order to see the efficacy of discrimination of each typing method [12].

Ethical Consideration

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 National Institutes of Health, University of the Philippines, Manila, Philippines

RESULTS

A total of 616 TB symptomatics were seen at the City of Santa Rosa City Health Office and Barangay Health Stations during the study period. 584 patients consented to participate and submitted sputum samples. Out of this, 129 patients had positive *M. tuberculosis* by culture. However, only 124 isolates underwent molecular typing since the 5 samples did not have adequate DNA for analysis. After molecular typing, further 8 strains were excluded from the 124 because of mixed infections. Double alleles were detected in two or more VNTR loci suggesting co-existence of different strains in the sample possibly due to contamination[13]. There is now a total of 116 isolates of *M. tuberculosis* for analysis.

Patient Characteristics

More than half (72 / 116, 62.1%) of the culture-positive patients were men. About 41.3% (48 / 116) were aged between 18 to 29 years. 31 / 116 or 26.7% had a high bacillary load based on a smear positivity of +3 or greater, and 41 / 116 or 35.3% of the patients had severe pulmonary TB, i.e., with pulmonary involvement with cavitation, miliary TB, extensive involvement of one lung or both lungs, or pleural effusion, as defined by chest X-ray findings. (Table 1) These patient characteristics well represent the TB patients of the Philippines in general, as compared with the national notification data.

Spoligotyping

Spoligotyping of the 116 *M. tuberculosis* isolates yielded 27 genotypes, and 22 of these genotypes were unique in the data set. 5 clusters were identified involving 94 strains, which means that the clustering rate was 81% [(94-5) /116 = 76.7%]. According to the spoligo-International Types (SIT), SIT 19 predominated with 81/116 (69.8%), followed by 7/116 (6.0%) of SIT 758, 2/116 (1.7%) each of SIT 1490, SIT 483 and SIT 1479, and one strain each of SIT 894, SIT 1169, SIT 287, SIT 897, SIT 1189, SIT 17, SIT 1247 and SIT 11. Fourteen strains were unclassified (12.1%). Of these clusters based on SIT, 19, 758, 1490, 483, 894, 1169, 287 and 897 comprise a sublineage of EAI2_Manilla clade, and thus this sublineage has a total of 96 strains (83%). The distribution of the different SITs is indicated in the Table 2.

The frequencies of EA12_Manilla strains out of all strains were similar across sexes, disease severity categories and bacillary load categories; EA12_Manilla accounts for 81.9% and 94.0% in males and females, 84.0% and 80.5% in not severe and severe groups, and 82.4% and 83.9% in low and high bacterial load groups, respectively (Table 3).

Based on the comparison of SIT 19 and other SIT strains, no significant difference in the frequency of SIT was seen for patient characteristics i.e., sex, disease severity and bacterial burden. SIT 19 was seen in 65.3% of male patients vs 77.3% of female patients, 69.4% of not severe cases

vs 70.7% of severe cases, and 69.4% of low bacterial burden cases vs 71.0% of high burden cases, respectively.

Typing of strains and clustering analysis by 15 MIRU-VNTR

Using the 15 MIRU-VNTR typing method followed by UPGMA dendrogram analysis, 69 different genotypes were identified, comprising 16 clusters formed by 63 isolates and 53 unique genotypes. Each cluster had 20, 10, 6, and 3 members, and 11 clusters had 2 members. The Hunter-Gaston Discriminative Index is calculated as 0.960. When spoligotyping and 15 MIRU-VNTR were simultaneously applied, 83 genotypes were identified with 10 clusters (each having 16, 9 and 4 members, and another 7 clusters had 2 members each.) involving 43 isolates. 73 genotypes were found to be unique. The HGDI was 0.975 (Figure 1, Table 5).

The patient characteristics, i.e., age, sex, disease severity and bacterial load, are not significant predictors for determining an infection with strains of clusters with 15 MIRU-VNTR genotype (Table 4). The strains clustered with combined 15 MIRU-VNTR and spoligotyping method show no significant association with the patient characteristics.

As anticipated from the univariate analysis, multiple logistic regression analysis revealed no significant predictor for determining an infection with EA12_Manilla as revealed by spoligotyping (Table 6). Also, these patient characteristics are not significantly associated with whether or not any

strain belongs to SIT 19-cluster as revealed by 15 MIRU-VNTR genotyping, with p values of 0.259, 0.135, 0.673, and 0.733, respectively.

DISCUSSION

Spoligotyping analysis showed that majority (96 / 116, 82.8 %) of the *M. tuberculosis* isolates seen in the city of Santa Rosa belonged to the EAI2_Manilla clade of the SpolD4. The other clades (U, LAM2, EAI3_IND and MANU2) constituted only a minority. 14 / 116 (12.1%) did not belong to a known clade. No strain belonging to the Beijing clade was identified. This is consistent with a previous study that involved also Philippine *M. tuberculosis* isolates that resulted in the creation of the Manila Family or EAI2_Manilla clade5). Based on published literature, the EAI family is prevalent in Southeast Asia, mainly in the Philippines, in Myanmar[14)] and Malaysia[15)]. Other studies have also shown that the EAI2_Manilla clade was also identified in other countries where large Filipino immigrant communities are located[16)17)18)19)]. The family is defined as an ancestral strain, containing the TbD1 region, and all isolates share the same spoligotype[9)20)].

The predominance of the EAI2_Manilla Family in the Philippines and among patients of Filipino descent may suggest the stability of the EAI2_Manilla genome by virtue of the innate properties of the bacteria and interaction with the host. Well conserved genotypes seem to prevail in areas with high incidence of tuberculosis [21)] such as the Philippines. Some genotypes have also been shown to

be more transmissible than others.[22)23)] Some genotypes of *M. tuberculosis* can be more capable of causing disease affecting particular organs[24)25)].

There have been some studies suggesting the possibility that BCG may have selected the particular prevalent genotypes. Anh *et al* suggested that the Beijing genotype was less associated with BCG, so that it may have resulted in the predominance of this genotype in Vietnam where BCG vaccination had been extensively used[26)27)]. A similar relationship between the prevalence of the Beijing strain and BCG vaccine coverage has also been shown in Tunisia and Ethiopia[28)]. Thus, it is possible that the Manila family is less sensitive to BCG vaccination and survived the high coverage of BCG, e.g., 84% in 2011[29)]. This is merely a possible hypothesis for the prevalence of the Manila family and remains to be determined.

Another possible hypothesis for the predominance of EAI2_Manilla strain is because EAI strains are better adapted for growth and transmission in high-temperature environments, but this also remains to be determined[30)].

In this study, possible association between patient demographic factors and the EAI2_Manilla clade and SIT 19 was also analyzed. No significant association was shown between these predominant genotypes and patient characteristics such as age, sex, disease severity and bacillary load. However, further studies should be made to elucidate the epidemiological, pathological and clinical characteristics of these genotypes in their diversity [31)32)].

Different mycobacterial strains may have differences as far as virulence and mechanisms of disease are concerned. These differences may have variable effects on smear positivity and clinical presentation as well as severity of TB. For example, there are several reports describing apparently enhanced in vivo virulence of certain members or sublineages of the "Beijing" lineage[33)34)35)36)]. The pathogenetic mechanism responsible for this is the production of a complex phenolic glycolipid which inhibits release of pro-inflammatory cytokines by macrophages[37)38)].

Apart from the Beijing genotype, there is a paucity however, of studies that describe phenotypic properties of the other TB lineages such as EAI. In one study done in Montreal, Canada, there was evidence to show that the East African-Indian lineage strains were associated with a lower risk of transmission and, possibly, a lower risk of developing severe forms of active disease[39)].

Among the 116 isolates we analyzed in our study, 43 / 116 (37.1%) of isolates would be considered to be potentially clustered in 10 groups based on the simultaneous spoligotyping and15 MIRU-VNTR. This means that 33 (43 – 10) patients may be due to recent transmission of infection, as many previous studies have shown correlation between the level of clustering and the proportion of disease due to recent transmission[11)40)41)42)43)].

Tuberculosis may result from recent infection or from reactivation of a latent infection acquired from the past. Based on the literature, recent infection is suspected if disease occurs within 5 years of infection and reactivation of a latent infection if disease occurs more than 5 years from

infection[44]. Since most of the affected individuals in the city of Santa Rosa were relatively young, there is more likely recent transmission. Using the (n-1) method, the recent transmission rate is 28.4% as above, based on the most precise typing system. However, a longer period of continuing observation as well as epidemiological analysis on the links should be done to analyze ongoing transmission. The rate of molecular clustering has been observed to increase over longer periods because transmission chains are more efficiently covered[45]. Because there was no association between age and clustering, the observed clustering could not be simply explained by the recent transmission. The possibility of the roles and exogenous reinfection and/or existence of predominant or endemic genotypes could not be excluded[46][47]. More detailed epidemiological information of the patients, such as situation of links among clustered patients, should be collected and analysed.

CONCLUSION

The predominant genotype of *M. tuberculosis* infecting the population of the city of Santa Rosa, a suburban community in the Philippines is the EAI2_Manilla family. This is consistent with previously published studies on the common clades of *M. tuberculosis* in the metropolitan area of the Philippines. Most of the TB patients affected are young, which suggests the possibility of recent tuberculosis transmission, as supported by the high clustering rate of 28%. No association was seen

between EAI2_Manilla clade and sex and age of patients. There was also no significant association seen between the EAI2_Manilla clade and bacillary load based on sputum–smear positivity and severity of pulmonary TB.

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# #

Table 1. Associations of Clade (EAI2_Manilla and other genotype strains) with Sex, Severity of pulmonary tuberculosis, and Bacillary Load of Patients with tuberculosis in the city of Santa Rosa, Laguna, the Philippines

		EAI2_Manilla		Others		p-value*
Total		96	82.8%	20	17.2%	
Age	18-29 years	41	85.4%	7	14.6%	0.524
	30 years+	55	80.9%	13	19.1%	
Sex	Male	59	81.9%	13	18.1%	0.766
	Female	37	84.1%	7	15.9%	
Severity	Not severe	63	84.0%	12	16.0%	0.632
	Severe	33	80.5%	8	19.5%	
Bacillary Load	Low	70	82.4%	15	17.6%	0.848
	High	26	83.9%	5	16.1%	

*p value for Pearson chi-square

[a] Severe pulmonary tuberculosis 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 Acid-fast bacilli (AFB) smear examination using Ziehl-Nielsen method. Low bacillary load is < or = AFB +2 or positive only for culture. High bacillary load is > or = AFB +3

Table 2. Distribution of *Mycobacterium tuberculosis* isolates according to sublineages designated by spoligotype from the city of Santa Rosa, Laguna, the Philippines (n = 116)

SIT (*1)	Sublineage (*2)	Spoligotype octal	Spoligotype binary	Number of isolates
19	EAI2 MANILLA	677777477413771		81
758	EAI2 MANILLA	677777477413700		7
1479	U	674777470001771		2
1490	EAI2 MANILLA	677767477413771		2
483	EAI2 MANILLA	677777477413701		2
1247	MANU2	777777607763771		1
287	EAI2 MANILLA	677777477413751		1
894	EAI2 MANILLA	677777477413731		1
1189	U	677777477403771		1
17	LAM2	677737607760771		1
1169	EAI2 MANILLA	677737477413771		1
897	EAI2 MANILLA	676003477413771		1
11	EAI3 IND	477777777413071		1
NA	unknown	777777607600171		1
NA	unknown	677777477553771		1
NA	unknown	677777477513771		1
NA	unknown	677777477400711		1
NA	unknown	677777470003771		1
NA	unknown	677777460000001		1
NA	unknown	677763477413771		1
NA	unknown	677757477413771		1
NA	unknown	677737607740771		1
NA	unknown	674003470003771		1
NA	unknown	667777477413771		1
NA	unknown	647777477413771		1
NA	unknown	617777477413771		1
NA	unknown	000000000000071		1

*1: SIT designates spoligotypes shared by two or more patient isolates in SpolDB4;"NA" designates spoligotypes not registered in SpolDB4

*2: Sublineages were designated according to SpolDB4. Spoligotypes not registered in SpolDB4 were designated as "unknown" sublineage.

Table 3. Associations of SIT 19 and other SIT with Sex, Severity of Pulmonary Tuberculosis, and Bacillary Load of Patients with tuberculosis in the city of Santa Rosa, Laguna, the Philippines

		SIT 19		Others		p-value*
Total		81	69.8%	35	30.2%	
Age	18-29 years	34	70.8%	14	29.2%	0.843
	30 years+	47	69.1%	21	30.9%	
Sex	Male	47	65.3%	25	34.7%	0.172
	Female	34	77.3%	10	22.7%	
Severity	Not severe	52	69.3%	23	30.7%	0.875
	Severe	29	70.7%	12	29.3%	
Bacillary Load	Low	59	69.4%	26	30.6%	0.872
	High	22	71.0%	9	29.0%	

*Pearson chi-square

[a, b] See footnote to Table 1.

Table 4. Associations of the Combined Spoligotyping and 15 loci of variable number of tandem repeats of mycobacterial interspersed repetitive units (15 MIRU-VNTR) typing-based Clustering and Age, Sex, Severity of Pulmonary Tuberculosis, and Bacillary Load of Patients in the city of Santa Rosa, Laguna, the Philippines

		Any cluster		Unique		p-value*
Total		73	62.9%	43	37.1%	
Age	18-29 years	29	60.4%	19	39.6%	0.638
	30 years+	44	64.7%	24	35.3%	
Sex	Male	44	61.1%	28	38.9%	0.604
	Female	2	11.8%	15	88.2%	
Severity	Not severe	47	64.4%	26	35.6%	0.673
	Severe	26	60.5%	17	39.5%	
Bacillary Load	Low	48	64.0%	27	36.0%	0.747
	High	25	61.0%	16	39.0%	

*Pearson chi-square

[a, b] See footnote to Table 1.

Table 5 Discriminatory ability of spoligotyping and 15 loci of variable number of tandem repeats of mycobacterial interspersed repetitive units (15 MIRU-VNTR) for *Mycobacterium tuberculosis* isolates from the city of Santa Rosa, Laguna, the Philippines

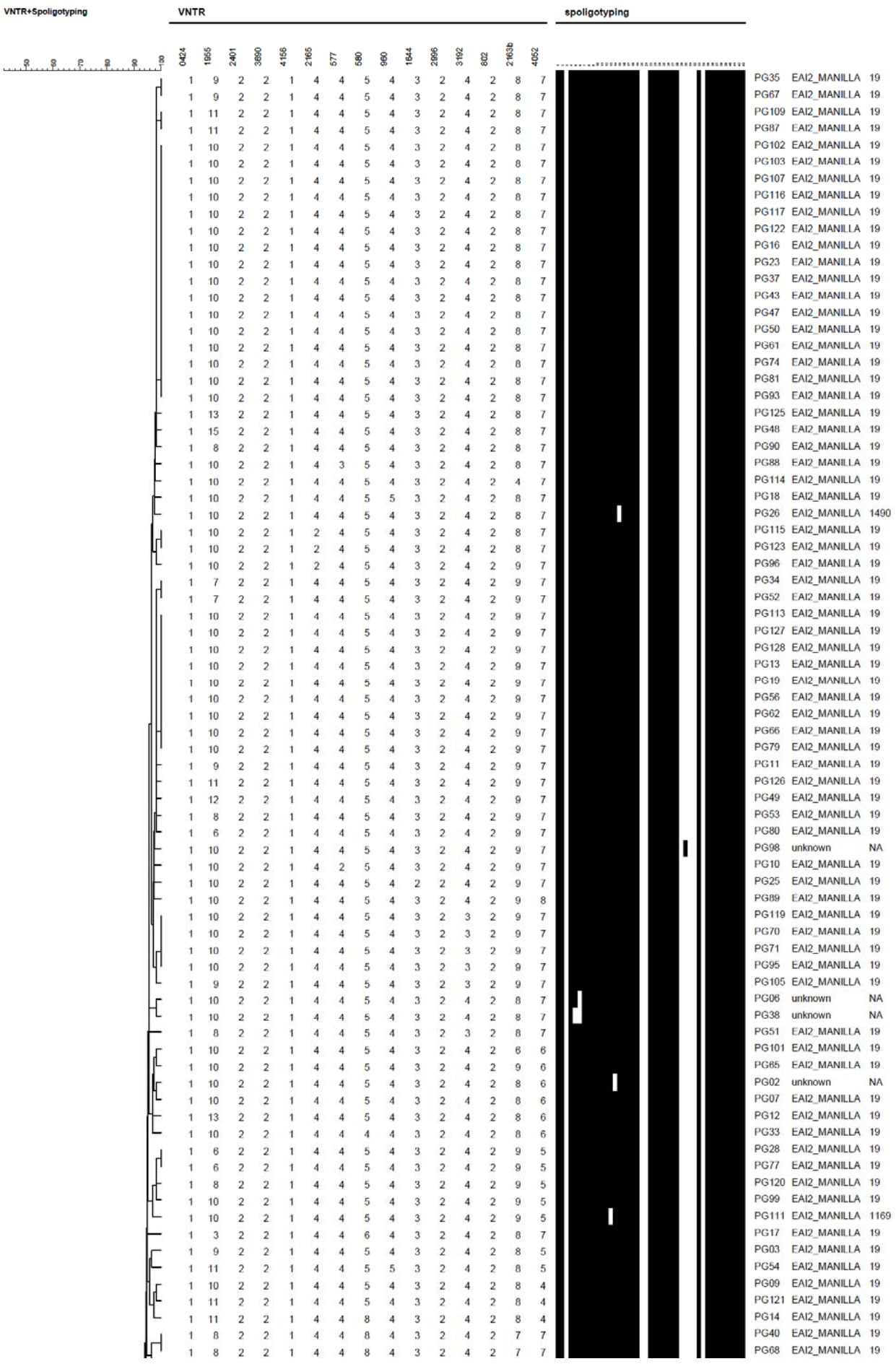
	Spoligotyping	15 MIRU-VNTR*	Spoligotyping and 15 MIRU-VNTR combined
HGDI[a]	0.511	0.960	0.975
Number of clusters	5	16	10
Number of genotypes	27	69	83
Number of clustered isolates	94	63	43
Clustering rate (%)[b]	76.7	40.5	28.4
Number of unique strains	22	53	73

*15 MIRU-VNTR include MIRU 4, 10, 16, 26, 31, 40; Mtub 04, 21, 30, 39; ETR A, C; and QUB-11b, 26, 4156

[a] HGDI: Hunter-Gaston Discrimination index

[b] Clustering rate is defined as $(N_c - n_c) / No$, where No 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

Figure 1. Unweighted Pair Group Method using Mathematical Averages (UPGMA) dendrogram (first column) based on composite data set (15 MIRU-VNTR)-Spoligotyping on the clinical isolates from tuberculosis patients in the city of Santa Rosa, Laguna, Philippines. (identification number : last column) Main clades are also annotated right to identification number.


VNTR+Spoligotyping
VNTR
spoligotyping
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50 60 70 80 90 100
PG35 EAI2_MANILLA 19
PG67 EAI2_MANILLA 19
PG109 EAI2_MANILLA 19
PG87 EAI2_MANILLA 19
PG102 EAI2_MANILLA 19
PG103 EAI2_MANILLA 19
PG107 EAI2_MANILLA 19
PG116 EAI2_MANILLA 19
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PG11 EAI2_MANILLA 19
PG126 EAI2_MANILLA 19
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PG53 EAI2_MANILLA 19
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PG98 unknown NA
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PG25 EAI2_MANILLA 19
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PG119 EAI2_MANILLA 19
PG70 EAI2_MANILLA 19
PG71 EAI2_MANILLA 19
PG95 EAI2_MANILLA 19
PG105 EAI2_MANILLA 19
PG06 unknown NA
PG38 unknown NA
PG51 EAI2_MANILLA 19
PG101 EAI2_MANILLA 19
PG65 EAI2_MANILLA 19
PG02 unknown NA
PG07 EAI2_MANILLA 19
PG12 EAI2_MANILLA 19
PG33 EAI2_MANILLA 19
PG28 EAI2_MANILLA 19
PG77 EAI2_MANILLA 19
PG120 EAI2_MANILLA 19
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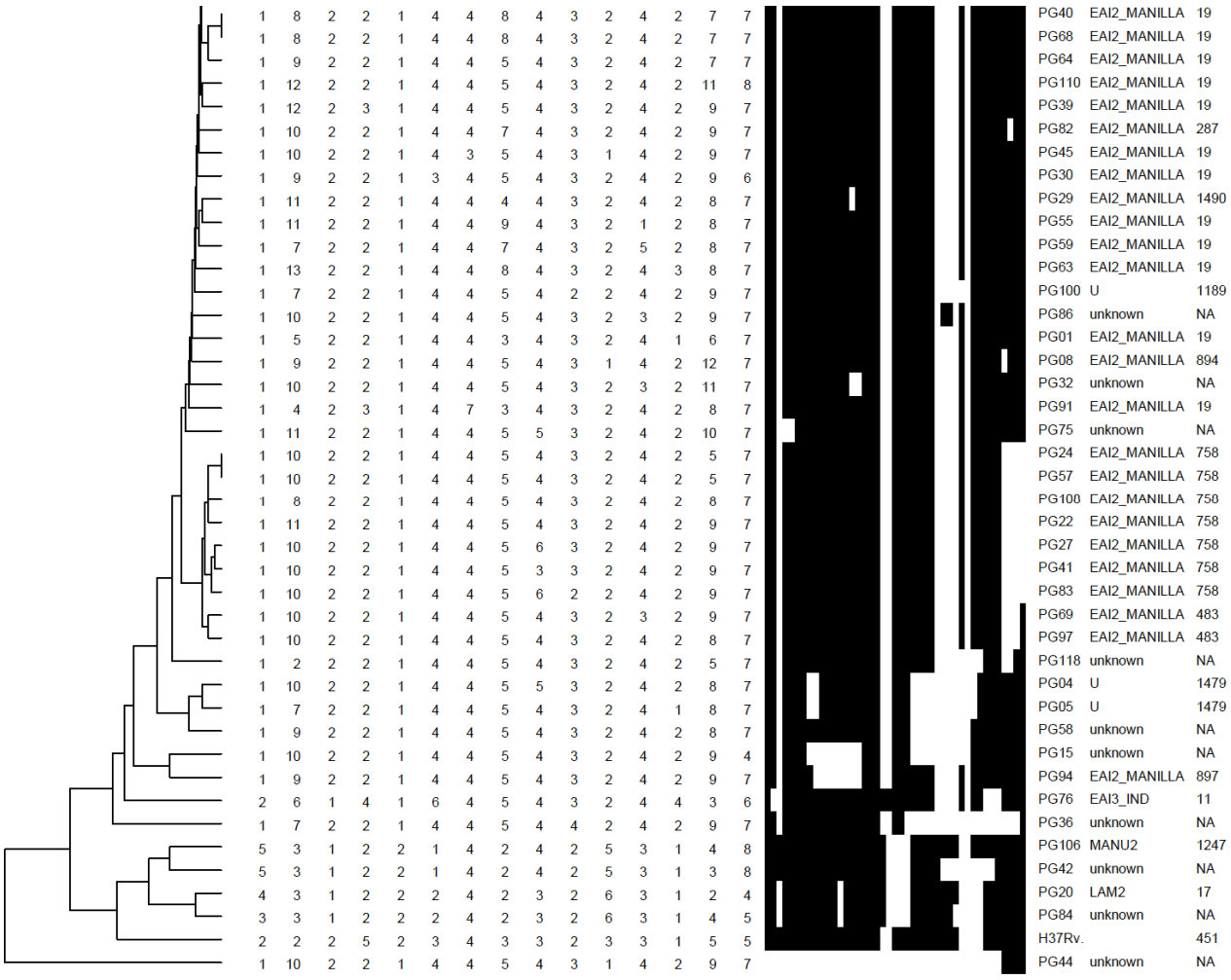
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1 9 2 2 1 4 4 5 4 3 2 4 2 7 7 PG64 EAI2_MANILLA 19
1 12 2 2 1 4 4 5 4 3 2 4 2 11 8 PG110 EAI2_MANILLA 19
1 12 2 3 1 4 4 5 4 3 2 4 2 9 7 PG39 EAI2_MANILLA 19
1 10 2 2 1 4 4 7 4 3 2 4 2 9 7 PG82 EAI2_MANILLA 287
1 10 2 2 1 4 3 5 4 3 1 4 2 9 7 PG45 EAI2_MANILLA 19
1 9 2 2 1 3 4 5 4 3 2 4 2 9 6 PG30 EAI2_MANILLA 19
1 11 2 2 1 4 4 4 4 3 2 4 2 8 7 PG29 EAI2_MANILLA 1490
1 11 2 2 1 4 4 9 4 3 2 1 2 8 7 PG55 EAI2_MANILLA 19
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1 10 2 2 1 4 4 5 4 3 2 3 2 11 7 PG32 unknown NA
1 4 2 3 1 4 7 3 4 3 2 4 2 8 7 PG91 EAI2_MANILLA 19
1 11 2 2 1 4 4 5 5 3 2 4 2 10 7 PG75 unknown NA
1 10 2 2 1 4 4 5 4 3 2 4 2 5 7 PG24 EAI2_MANILLA 758
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1 8 2 2 1 4 4 5 4 3 2 4 2 8 7 PG100 EAI2_MANILLA 750
1 11 2 2 1 4 4 5 4 3 2 4 2 9 7 PG22 EAI2_MANILLA 758
1 10 2 2 1 4 4 5 6 3 2 4 2 9 7 PG27 EAI2_MANILLA 758
1 10 2 2 1 4 4 5 3 3 2 4 2 9 7 PG41 EAI2_MANILLA 758
1 10 2 2 1 4 4 5 6 2 2 4 2 9 7 PG83 EAI2_MANILLA 758
1 10 2 2 1 4 4 5 4 3 2 3 2 9 7 PG69 EAI2_MANILLA 483
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2 6 1 4 1 6 4 5 4 3 2 4 4 3 6 PG76 EAI3_IND 11
1 7 2 2 1 4 4 5 4 4 2 4 2 9 7 PG36 unknown NA
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3 3 1 2 2 2 4 2 3 2 6 3 1 4 5 PG84 unknown NA
2 2 2 5 2 3 4 3 3 2 3 3 1 5 5 H37Rv. 451
1 10 2 2 1 4 4 5 4 3 1 4 2 9 7 PG44 unknown NA

Table 6. Logistic regression analysis of patient characteristics for association with EAI2_Manilla clade

Characteristics	95% CI for OR			p-value
	OR	Lower	Upper	
Age	0.978	0.943	1.014	0.232
Sex	0.808	0.285	2.291	0.689
Severity of PTB	1.150	0.415	3.188	0.788
High bacillary load*	0.870	0.276	2.742	0.812

OR: odds ratio, CI: confidence interval, PTB: pulmonary tuberculosis

* High bacillary load is sputum smear positivity of > or = AFB +3

和文抄録#

フィリピン一郊外地域住民結核患者から得られた結核菌の分子疫学的分析#

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背景： フィリピンはWHOの指定する結核高負担国のひとつである。郊外都市であるラグナ州サンタ・ロサの町の診療所を受診した結核患者から得た結核菌の分子疫学的文政を行った。方法：総数116株の結核菌について遺伝子型の分析をスポリゴタイピング、15個の座位を用いたVNTR法によって実施した。菌株はスポリゴタイプ国際データベース（SPolDB4）と比較した。クラスター分析はスポリゴタイピングおよびVNTRを用いて行った。結果：患者の多くは若年者（18〜29歳が41.4%）で男性が多かった（62.1%）。74%が塗抹陽性、また37%は重症例であった。遺伝子型をSpolDB4と比較すると14種のSIT型が見いだされ、そのなかではSIT19が最も多かった（81株70%）。14株（12%）はSpolDB4にみられない型であった。主要抗酸菌系統の分布をみるとEAI2_Manilla (96株, 82.8%)、U 3（3株, 2.6%）、以下LAM2、EAI3_IND、MANU2が 1 株(0.9%)であった。単変量および多変量解析によってEAI2_Manilla系統やSIT型と患者背景要因(性、年齢、排菌程度、重症度)の関連を分析したがいずれも有意の関連はみられなかった。タイピング方法別にクラスター形成をみると、スポリゴタイピングでは94株が5個のクラスターを形成、ハンター・ガストン判別指数（HGDI）は0.511、15 MIRU-VNTR では63株が16個のクラスターを形成、HGDIは 0.960、またスポリゴタイピングと15 MIRU-VNTRを組み合わせた場合には43株が10クラスターを作り、HGDIは0.975であった。この地域での患者の多くが若年者であることから、感染伝播率が高いことは想定される。しかし患者年齢とクラスター形成の間に有意の関連がみられず、観察された高いクラスター形成の解釈についてはさらなる分子的および疫学的研究を要する。結論：この研究はフィリピンにおいて系統的かつ前向きに患者

標本を集めて結核菌の遺伝子型をみた最初の分子疫学研究である。ラグナ州サンタロサの患者集団の多くはEAI2_Manilla系統に属する菌株に感染していることが知られた。最近の感染伝播の割合は高く、より効果的で早期の診断と十分な治療の必要性を物語っている。