

## *Mycobacterium tuberculosis* Drug Resistance and Transmission among Human Immunodeficiency Virus–Infected Patients in Ho Chi Minh City, Vietnam

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**Abstract.** Vietnam has a high burden of tuberculosis (TB) and multidrug-resistant (MDR) TB, but drug resistance patterns and TB transmission dynamics among TB/human immunodeficiency virus (HIV) coinfecting patients are not well described. We characterized 200 *Mycobacterium tuberculosis* isolates from TB/HIV coinfecting patients diagnosed at the main TB referral hospital in Ho Chi Minh City, Vietnam. Phenotypic drug susceptibility testing (DST) for first-line drugs, spoligotyping, and 24-locus mycobacterial interspersed repetitive unit (MIRU-24) analysis was performed on all isolates. The 24-locus mycobacterial interspersed repetitive unit clusters and MDR isolates were subjected to whole genome sequencing (WGS). Most of the TB/HIV coinfecting patients were young (162/174; 93.1% aged < 45 years) males (173; 86.5% male). Beijing (98; 49.0%) and Indo-Oceanic (70; 35.0%) lineage strains were most common. Phenotypic drug resistance was detected in 84 (42.0%) isolates, of which 17 (8.5%) were MDR; three additional MDR strains were identified on WGS. Strain clustering was reduced from 84.0% with spoligotyping to 20.0% with MIRU-24 typing and to 13.5% with WGS. Whole genome sequencing identified five additional clusters, or members of clusters, not recognized by MIRU-24. In total, 13 small (two to three member) WGS clusters were identified, with less clustering among drug susceptible (2/27; 7.4%) than among drug-resistant strains (25/27; 92.6%). On phylogenetic analysis, strains from TB/HIV coinfecting patients were interspersed among strains from the general community; no major clusters indicating transmission among people living with HIV were detected. Tuberculosis/HIV coinfection in Vietnam was associated with high rates of drug resistance and limited genomic evidence of ongoing *M. tuberculosis* transmission among HIV-infected patients.

### INTRODUCTION

Globally, *Mycobacterium tuberculosis* was the number one infectious disease killer in 2016, causing an estimated 1.3 million deaths.<sup>1</sup> Tuberculosis (TB) is also the leading cause of death among people living with human immunodeficiency virus (HIV). Of the estimated 10.4 million new TB cases that occurred worldwide in 2016, more than one million (11%) were coinfecting with HIV, with 370,000 TB-related deaths in this group.<sup>1</sup> Large numbers of patients with multidrug-resistant (MDR; resistance to at least both isoniazid and rifampicin) TB, and potential epidemic spread of these drug-resistant strains, pose a particular challenge.<sup>2,3</sup>

In 2016, Vietnam was ranked 16th among high-burden TB countries and 13th among high-burden MDR-TB countries in the world.<sup>1</sup> Multidrug-resistant TB was reported in 4.1% of new and 26% of retreatment TB cases.<sup>1</sup> Multidrug-resistant TB can be acquired by patients initially infected with a drug-susceptible strain, usually as a result of inadequate treatment (defined as secondary resistance), or occur following infection with an MDR strain (primary or transmitted drug resistance).<sup>4</sup> A common, but misplaced, assumption has been that MDR-TB among new cases provides an accurate reflection of transmitted drug resistance.<sup>5</sup> However, it has been demonstrated that many retreatment cases with MDR-TB also represent transmitted drug resistance.<sup>6</sup>

The epidemic spread of MDR-TB is a particular concern in areas with poor control of ongoing TB transmission, especially

among HIV-infected patients with severe immunodeficiency where health-care facilities can serve as epidemic amplifiers.<sup>7,8</sup> Global TB control efforts have been complicated by increased drug resistance and the negative synergism of TB and HIV coinfection.<sup>8</sup> Despite the rapid expansion MDR-TB services and national efforts to identify and treat MDR-TB cases in Vietnam, case numbers continue to rise.<sup>10</sup> Furthermore, MDR-TB/HIV coinfection often leads to poor treatment outcomes,<sup>11</sup> and the risk of MDR-TB transmission within settings that provide HIV care has been well documented.<sup>12</sup>

Of concern, TB/HIV coinfection in Vietnam has been associated with high rates of drug resistance,<sup>13</sup> but the genetic determinants of drug resistance remain poorly described and a better understanding of TB transmission dynamics is required to guide prevention strategies. Traditional methods of *M. tuberculosis* genotyping, such as spoligotyping or 24-locus mycobacterial interspersed repetitive unit (MIRU-24) analysis, have been hampered by poor discriminatory power.<sup>14</sup> Whole genome sequencing (WGS) offers unprecedented strain resolution, especially of lineage 2 (Beijing) strains which is the dominant lineage circulating in Vietnam.<sup>15</sup> It also provides genome-wide view for assessment of drug resistance mutations. This study aimed to examine the transmission dynamics and drug resistance-conferring mutations in *M. tuberculosis* strains isolated from TB/HIV coinfecting patients in Vietnam by using a variety of contemporary molecular methods, including WGS.

### METHODS

We conducted a retrospective laboratory-based analysis of 200 *M. tuberculosis* isolates from TB/HIV coinfecting patients in Ho Chi Minh City, Vietnam. Consecutive culture-positive isolates identified at the Pham Ngoc Thach Hospital

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TABLE 1

Demographic characteristics and drug resistance profiles of TB/HIV coinfecting patients in Ho Chi Minh City, Vietnam

Characteristic (N = 200)	n (%)	
Gender		
Male	173 (86.5)	
Age distribution (N = 174)		
Median age (range) (years)	31 (17–57)	
Age category		
17–24	13 (7.5)	
25–34	111 (63.8)	
35–44	38 (21.8)	
≥ 45	12 (6.9)	
Strain lineages*		
Lineage 1 (Indo-Oceanic; East African-Indian, EAI)	70 (35.0)	
Lineage 2 (East Asian; Beijing)	98 (49.0)	
Lineage 3 (Indian/Central Asian; Delhi/CAS)	0 (0)	
Lineage 4 (Euro-American, EA)	22 (11.0)	
Other and unknown†	10 (5.0)	
Drug resistance profiles	Phenotypic	Genotypic‡
Any drug resistance	84 (42.0)	82 (41.0)
Resistance to a single drug (mono-resistance)	37 (18.5)	31 (15.5)
H	4 (2.0)	5 (2.5)
R	0	0
E	0	0
S	34 (17.0)	25 (12.5)
Z	NA	0
Resistance to multiple drugs (but not MDR)	29 (14.5)	32 (16.0)
HS	28 (14.0)	20 (10.0)
RS	1 (0.5)	0
HZ	NA	2 (1.0)
SZ	NA	2 (1.0)
SQ	NA	2 (1.0)
HSZ	NA	4 (2.0)
HSEZ	NA	2 (1.0)
Multidrug resistant (MDR)	17 (8.5)	20 (10.0)
HR	0	0
HRS	7 (3.5)	2 (1.0)
HRSE	10 (5.0)	8 (4.0)
HRSZ	NA	1 (0.5)
HRSEZ	NA	9 (4.5)

E = ethambutol; H = isoniazid; HIV = human immunodeficiency virus; MDR = resistant to at least H and rifampicin; NA = not applicable, resistance to Z and Q not assessed; Q = quinolones; R = rifampicin; S = streptomycin; TB = tuberculosis; Z = pyrazinamide.

\* TB lineages classification as of Gagneux and Small.<sup>16</sup>

† Spoligo and 24-locus mycobacterial interspersed repetitive unit typing unsuccessful.

‡ All phenotypic drug-resistant isolates were sequenced—no resistance mutations were detected in two S mono-resistant strains.

Mycobacterial Reference Laboratory, during periods that no other studies were performed (2009–2010 and then again in 2014), were subjected to phenotypic drug susceptibility testing (DST), spoligotyping, MIRU-24, and WGS. Basic demographic data were acquired from the laboratory database. Whole genome sequencing data were compared with publicly accessible sequences from a recent study by Holt et al.<sup>17</sup> that documented strain diversity in HIV-uninfected TB patients from Ho Chi Minh City in a similar time frame.

**Study setting.** Ho Chi Minh City is the most populous metropolis in Vietnam, with an estimated population of 8.2 million.<sup>18</sup> Pham Ngoc Thach Hospital is the largest (750-bed) lung disease treatment facility in Ho Chi Minh City and treated more than 7,600 TB patients in 2016. The hospital serves as a tertiary referral center for TB patients and people living with HIV throughout southern Vietnam, treating around 350 HIV-infected (including TB/HIV coinfecting)

patients per year.<sup>18</sup> The hospital also hosts a World Health Organization–accredited regional mycobacterial reference laboratory.<sup>19</sup>

**Drug susceptibility testing.** Primary mycobacterial cultures were grown on solid Lowenstein–Jensen (LJ) media (bioMérieux SA, Marcy-l'Étoile, France) or liquid culture (BACTEC™ or MGIT™ 960; Becton Dickinson & Co., Franklin Lakes, NJ). All isolates identified as *M. tuberculosis* underwent phenotypic DST against isoniazid, rifampicin, streptomycin, and ethambutol by the standard 1% proportion method.<sup>20</sup> Cultures were grown on solid LJ media with the following critical drug concentrations: isoniazid (0.2 µg/mL), rifampin (40 µg/mL), streptomycin (4 µg/mL), and ethambutol (2 µg/mL).

**Twenty four-locus mycobacterial interspersed repetitive unit and spoligotyping.** Spoligotyping was performed according to the standard protocol<sup>21</sup> and phylogenetic lineages were assigned using the SIVITWEB ([http://www.pasteur-guadeloupe.fr:8081/SITVIT\\_ONLINE/](http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/)) and SPOTCLUST (<http://cgj2.cs.rpi.edu/~bennek/SPOTCLUST.html>) databases. The 24-locus mycobacterial interspersed repetitive unit typing was also performed as described before<sup>22</sup> and strain lineage assigned using the <http://www.miru-vnrplus.org> online database, which was also used to construct the minimum spanning tree. Two or more isolates sharing an identical MIRU-24 profile were considered a cluster.

**Whole genome sequencing and bioinformatics analysis.** Genomic DNA from *M. tuberculosis* isolates were extracted as described before<sup>23</sup> from all drug-resistant and MIRU-24-clustered isolates. Libraries were constructed using Nextera XT DNA preparation kit (Illumina, San Diego, CA) and genome sequencing was performed on the NextSeq500 (Illumina) at the Center for Infectious Diseases and Microbiology, New South Wales Health Pathology in Sydney, Australia. Sequencing reads were mapped to the reference genome H37Rv (Genbank NC\_000962) using the RedDog pipeline (<https://github.com/katholt/RedDog>). An initial phylogenetic tree was inferred using FastTree v2.1.8.<sup>24</sup> Variant calling was performed using Snippy version 3.1 (<https://github.com/tseemann/snippy>) and careful checks performed for low-frequency variants in the case of discrepant phenotypic and genotypic DST results. Insertions and deletions (indels) were assessed as possible markers of drug resistance, but were not included in the phylogenetic tree reconstruction. Genomes with ≤ 10 single nucleotide polymorphisms (SNP) differences (calculated as the number of SNP difference between individual members of the cluster compared with the ancestral node) were classified as a WGS cluster, and those with 11–20 SNPs as a “possible cluster,” indicative of recent transmission.<sup>15</sup>

Maximum likelihood phylogenetic trees were constructed using RAxML (version 8.2; Heidelberg, Germany) and FastTree (version 2.1.8) using the GTR model of nucleotide substitution and a Gamma model of rate heterogeneity to analyze a concatenated alignment of SNP alleles. Trees were visualized using Microreact ([www.microreact.org](http://www.microreact.org)) and Figtree v1.4.3. Ancestral sequence reconstruction was performed using FastML (version 3.1).

**Statistical analysis and human research ethics approval.** Demographic and clinical characteristics of different *M. tuberculosis* lineages, as well as the odds ratio (OR) for clustering of drug susceptible and drug-resistant isolates, were examined using STATA version 13 (StataCorp,

College Station, TX). Branch length comparisons were generated using R package phytools v0.5 (The Free Software Foundation, Boston, MA). The product of branch length and the number of substitutions (i.e., SNPs) per genome, mapped to each terminal branch, provided the branch length for each isolate. The boxplots comparing branch lengths of lineage-specific phylogenetic trees for *M. tuberculosis* strains isolated from HIV-infected and HIV-uninfected patients were plotted using the *ggplot2* and metrics calculated using *stats* (R version 3.2.5) (The Free Software Foundation).<sup>25</sup> The *P* value for branch length comparison between *M. tuberculosis* strains isolated from HIV-infected and HIV-uninfected patients

was calculated with GraphPad prism version 7 (San Diego, CA) (using a hypothetical mean of 100).

The study was approved by the Pham Ngoc Thach Hospital Ethics Review Committee (approved on October 9, 2014) and the Human Research Ethics Committee of the University of Sydney (Project No 2015/522).

RESULTS

Table 1 provides an overview of the demographic characteristics and drug resistance profiles of TB/HIV coinfecting patients included in the study. The majority (63.8%) were

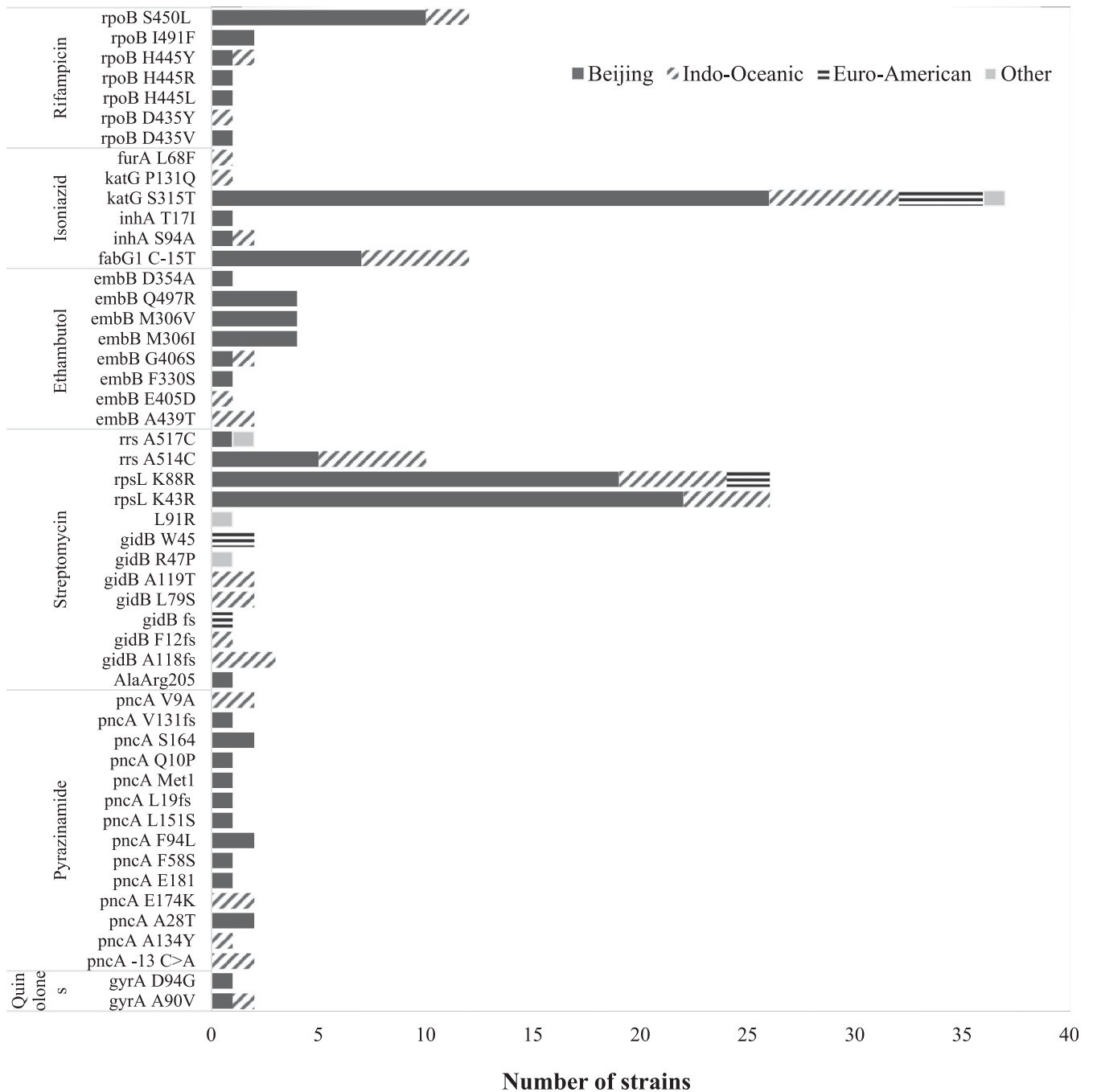


FIGURE 1. Drug resistance mutations identified in different *Mycobacterium tuberculosis* strain lineages.

young adults (25–34 years of age), predominantly (86.5%) male. Beijing (98; 49.0%) and Indo-Oceanic (70; 35.0%) lineage strains were most common. Phenotypic resistance against any of the first-line drugs was identified in 84 (42.0%) isolates; 17 (8.5%) were MDR by phenotypic and 20 (10.0%) by genotypic assessment. Patients with MDR-TB were also predominantly young (17/20; 85.0% < 45 years of age) males (14/20; 70.0%), infected with Beijing (14/20; 70.0%) or Indo-Oceanic (3/20; 15.0%) lineage strains. Among phenotypically drug-resistant strains, resistance to streptomycin was near universal (80/84; 95.2%), with the only exception being two isolates with isoniazid mono-resistance and two with isoniazid as well as pyrazinamide resistance. Phenotypically, 10/84 (11.2%) drug-resistant isolates were pan resistant to all first-line drugs tested (isoniazid, rifampicin, ethambutol, and streptomycin), of which nine displayed concurrent genotypic resistance to pyrazinamide. No extensively drug-resistant (XDR; MDR with additional resistance to a fluoroquinolone and second-line injectable drugs) or pre-XDR strains were identified by DST, but two streptomycin-resistant strains had *gyrA* mutations indicative of fluoroquinolone resistance.

Drug resistance-conferring mutations identified in relevant *M. tuberculosis* strain lineages are presented in Figure 1. Among phenotypically isoniazid-resistant isolates, the *katG* S315T mutation was most common (38/54; 70.3%) and found in all phylogenetic lineages, followed by the C-15T mutation in *fabG1-inhA* regulatory region (12/54; 22.2%). The most common mutation responsible for resistance to rifampicin was *rpoB* S450L (11/20; 55%). Most *rpoB* mutations (S450L, H445Y/R/L, and D435Y/V) were identified inside the rifampicin resistance-determining region (RRDR) of *rpoB* gene targeted by Xpert MTB/RIF<sup>®</sup>. One mutation was found outside the RRDR (I491F) in two unrelated TB-HIV isolates. Three phenotypically drug-resistant but non-MDR isolates carried mutations associated with a high likelihood of resistance to isoniazid and rifampicin; two phenotypically rifampicin-susceptible isolates had non-synonymous *rpoB* mutations (D435Y and I491F) and one isoniazid susceptible isolate had a *fabG1* C-15T *inhA* mutation (isolates 25, 31, and 56, Supplemental Table 1). Supplemental Table 1 summarizes results from phenotypic and genotypic resistance testing. Resistance-conferring mutations to ethambutol and pyrazinamide were highly variable with major discrepancies between phenotypic methods and susceptibility profiles

inferred from WGS. Phenotypic resistance to pyrazinamide was not assessed.

Table 2 describes and compares the number and size of clusters identified by spoligotyping, MIRU-24, and WGS. Overall, 84% of isolates were clustered by spoligotyping, including all (98; 100.0%) Beijing lineage strains, which share a unique spoligotyping profile. The 24-locus mycobacterial interspersed repetitive unit clustering was identified in 20.0% of isolates (29.6% of Beijing lineage strains), with 16 clusters consisting of two to four isolates per cluster. Whole genome sequencing confirmed the presence of 13 (27 isolates) clusters (Table 3) and three (seven isolates) “possible clusters” (characterized by 11–20 SNP difference between genomes). The variable clustering identified by MIRU-24 and WGS is further explored in Table 3. Among 16 MIRU-24 clusters, four (25.0%) matched with WGS clusters (including “possible clusters”), six (37.5%) had a partial match, and six (37.5%) had no match. Given the increased resolution of WGS, it is expected that clustering will generally be reduced compared with MIRU-24; however, WGS identified five new clusters (or members of clusters) that were not recognized by MIRU-24 (Table 3).

The comparison of *M. tuberculosis* genomes sequenced in this study identified 13 small (two to three strain) clusters dispersed among all the major strain lineages, with nearly all clusters (12/13; 92.3%) containing drug-resistant strains (Figure 2). We documented less clustering among drug-susceptible (2/27; 7.4%) than among drug-resistant strains (25/27; 92.6%) (OR 0.93, 95% confidence interval: 0.77–0.98). A combined analysis of our dataset and that of non-HIV-infected patients between 2008 and 2011 described by Holt et al.<sup>17</sup> demonstrated that the strains from TB/HIV coinfecting patients were intermingled with strains circulating in the general community (Figure 3). This comparison identified 21 clusters among HIV-infected and uninfected patients, including one big clonal outbreak with 17 isolates, four medium-sized clusters (5–10 isolates each), and 16 small clusters (two to four isolates each). Of the 102 sequenced strains from TB-HIV coinfecting patients, probable recent transmission was identified in 36 (25.3%), with most of the transmitted strains (25/36; 69.4%) appearing to be acquired in the community. Supplemental Table 2 presents the *M. tuberculosis* strain lineage, drug resistance profile, and cluster characteristics of strains isolated from TB/HIV coinfecting patients compared with those from the general community. All large clusters appeared to be independent of the patients’ HIV status.

TABLE 2  
Number and size of clusters identified by spoligotyping, MIRU-24, and WGS

Cluster detection	Spoligotyping	MIRU-24*	WGS†
Clustered isolates (N = 200)	168 (84.0%)	40 (20.0%)	27 (13.5%)
Number of clusters	16	16	13
Median cluster size (range)	2.5 (2–93)	3 (2–4)	2 (2–3)
SNP range	NA†	20–399	1–10
Clusters with multidrug-resistant isolates	6	6	3
Lineage-specific clustering			
Beijing (N = 98)	98 (100.0%)	29 (29.6%)	12 (14.3%)
Indo-Oceanic (N = 70)	53 (75.7%)	7 (10.0%)	9 (12.8%)
Euro-American (N = 22)	14 (63.6%)	4 (18.2%)	6 (27.3%)
Other (N = 10)	4 (40.0%)	0 (0%)	0 (0%)

MIRU-24 = 24-locus mycobacterial interspersed repetitive unit typing; SNP = single nucleotide polymorphisms; WGS = whole genome sequencing.

\* Only identical MIRU-24 profiles were recognized as clusters.

† Only MIRU-24 clusters and drug-resistant strains were sequenced—strains with ≤ 10 SNPs were identified as a cluster.

TABLE 3  
Variable clustering identified by MIRU-24 and WGS

ID	MIRU-24 clusters ID	MIRU-24 cluster* size (SNP difference)	WGS cluster ID	WGS cluster size (SNP difference)	SNP by WGS	MIRU-24 profile	Lineage
10-V027	Cluster 1	2 (0)	Cluster 1.WGS	2 (0)	0	244233342644425163353725	Beijing
10-V029					0	244233342644425163353725	
10-V109	Cluster 2	2 (4)	Cluster 2.WGS	2 (4)	1	224225132134425112333732	Euro-American
14-V120					3	224225132134425112333732	
14-V045	Cluster 3	2 (11)	Not clustered	Not clustered	5	244233352644425173354823	Beijing
14-V057					6	244233352644425173354823	
10-V004	Cluster 4	3 (16)	Not clustered	Not clustered	7	244233382A44425173355823	Beijing
14-V052					2	244233382644425173355823	
14-V054	Cluster 5	3 (21)	Not clustered	Not clustered	7	244233382644425173355823	Beijing
14-V078					7	244233342644425163353723	
14-V189	Cluster 3.WGS			2 (8)	11	244233342644425163353723	
14-V082					5	244233342644425163353723	
14-V064	Not clustered	Not clustered			3	244235342674425163353723	
10-V007	Cluster 6	2 (52)	Not clustered	Not clustered	NA	233213322534435163332632	Euro-American
14-V053					4	233213322534435163332632	
14-V055	Not clustered	Not clustered			1	233213322534435463332632	
14-V128	Cluster 7	2 (67)	Not clustered	Not clustered	NA	224624272273245422354613	Indo-Oceanic
14-V134					NA	224624272273245422354613	
10-V026	Cluster 8	2 (73)	Not clustered	Not clustered	NA	324634252273245423354613	Indo-Oceanic
10-V025					1	324634252273245423354713	
10-V028	Not clustered	Not clustered			1	244233342644425163353725	
10-V010	Cluster 9	3 (187)			1	324634252273245223A54613	
14-V079					NA	324634252273245223354613	
14-V154			Not clustered	Not clustered	NA	324634252273245223354613	
14-V034	Cluster 10	2 (89)	Not clustered	Not clustered	NA	244232352644425173353824	Beijing
09-V088					NA	244232352644425173353824	
14-V118	Cluster 11	2 (158)	Not clustered	Not clustered	NA	244233352644425172353823	Beijing
10-V131					NA	244233352644425172353823	
09-V085	Cluster 12	4 (172)	Not clustered	Not clustered	NA	244232352644425173353824	Beijing
14-V177					NA	244232352644425173353824	
14-V178			Not clustered	Not clustered	NA	244232352644425173353824	
10-V012			Cluster 6.WGS	2 (2)	0	2442323526444251733538A4	
14-V081	Not clustered	Not clustered			2	244232352642425173453824	
14-V074	Cluster 13	3 (194)	Cluster 7.WGS	2 (7)	2	244233352544425173353823	Beijing
14-V030					5	244233352544425173353823	
14-V083			Cluster 8.WGS	2 (1)	0	244233352544425173353823	
14-V060	Cluster 14	2 (291)	Not clustered	Not clustered	1	244233352544425173453823	Beijing
14-V037					NA	244233352544425163353723	
14-V046	Cluster 15	4 (238)	Not clustered	Not clustered	NA	244233352644425173353823	Beijing
14-V051					NA	244233352644425173353723	
14-V167			Not clustered	Not clustered	NA	244233352644425173353823	
14-V041			Not clustered	Not clustered	NA	244233352644425173353823	
10-V113	Cluster 16	2 (399)	Not clustered	Not clustered	NA	244233352544425172353823	Beijing
14-V146					NA	244233342544425172353223	
14-V034	Not clustered	No cluster by MIRU	Cluster 9.WGS	2 (7)	3	244232352644425173353824	Beijing
14-V039					4	244232352646425173353823	
14-V035	Not clustered	No cluster by MIRU	Cluster 10.WGS	2 (7)	3	222624392393246324A65411	Indo-Oceanic
14-V038					4	2226243A2393246424365411	
10-V006	Not clustered	No cluster by MIRU	Cluster 11.WGS	2 (3)	1	224633272273234223354813	Indo-Oceanic
14-V049					2	224633272273234423354813	
14-V044	Not clustered	No cluster by MIRU	Cluster 12.WGS	2 (5)	2	224534272AA3245223346613	Indo-Oceanic
14-V056					3	224514272AA4225223346613	
14-V040	Not clustered	No cluster by MIRU	Cluster 13.WGS	2 (0)	0	234232352274445223364624	Indo-Oceanic
14-V043					0	<b>2442333826446251733548A3</b>	

MIRU-24 = 24-locus mycobacterial interspersed repetitive unit typing; SNP = single nucleotide polymorphisms; WGS = whole genome sequencing.  
 \* MIRU-24 cluster identified as identical profiles and tree-based identification MIRU-VNTRplus (<http://www.miru-vntrplus.org/MIRU/index.faces>).  
 † WGS cluster identified as ≤ 10 SNPs difference, "possible cluster" as 10–20 SNPs difference, and "not clustered" > 20 SNPs difference.

Multidrug-resistant TB transmission was noted in a number of small clusters, but unrelated to HIV infection status. Supplemental Table 3 which includes all sequenced strains reflects 3/13 (23.0%) potential "crossover" clusters between the two time periods 2010 and 2014.

The average branch length of *M. tuberculosis* genomes derived from SNP-based phylogenetic trees in TB patients with and without HIV coinfection was used as a proxy measure for the recency of TB infection. The average

branch length in strains from HIV-infected patients was significantly shorter than those from HIV-uninfected patients ( $P < 0.01$ ). This observation was consistent across all phylogenetic lineages, but most pronounced in Indo-Oceanic strains (Figure 4). To assess the role of patients' age in these differences, given the young age of TB/HIV coinfecting patients, the same analysis was performed comparing HIV-infected patients in different age groups (Supplemental Figure 1). This demonstrated a pronounced

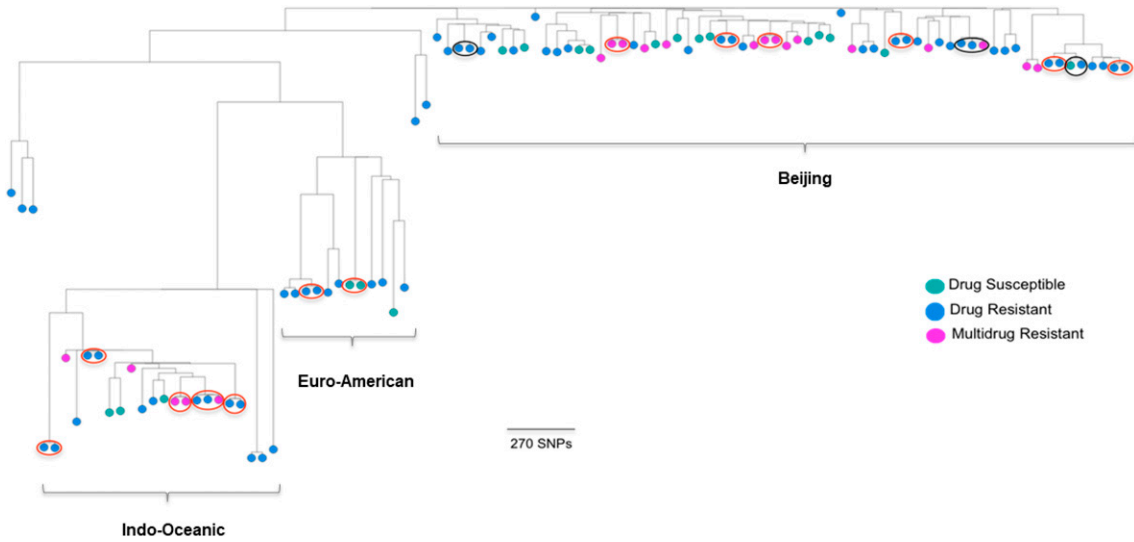


FIGURE 2. Phylogenetic tree of sequenced\* *Mycobacterium tuberculosis* strains from patients with tuberculosis/human immunodeficiency virus coinfection in Ho Chi Minh City, Vietnam. Multidrug resistant (MDR): resistant to at least isoniazid and rifampicin; \*All strains that were drug resistant (84) or clustered by 24-locus mycobacterial interspersed repetitive unit (40) were sequenced; Red circles indicate identified clusters; Black circles indicate possible cluster.

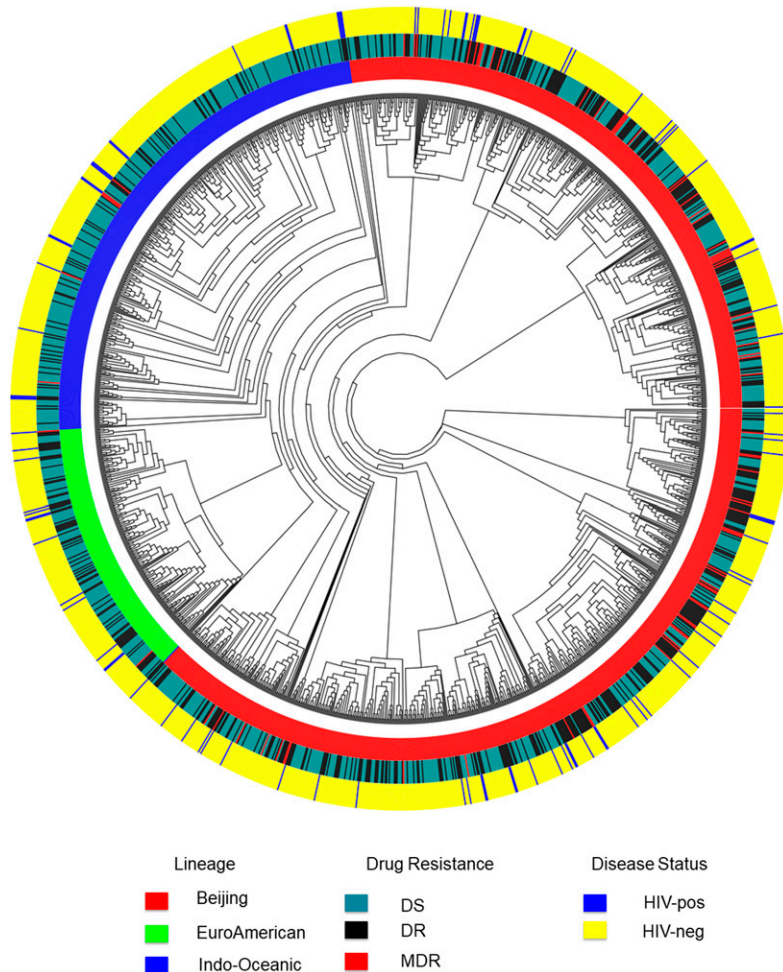


FIGURE 3. *Mycobacterium tuberculosis* strains identified in tuberculosis/HIV coinfecting patients in Ho Chi Minh City, Vietnam, compared with community strains identified among non-HIV-infected patients.\* DR = drug resistant; DS = drug susceptible; HIV = human immunodeficiency virus; MDR = multidrug resistant. \*Sequence data for community *M. tuberculosis* strains were derived from Holt et al.<sup>15</sup>



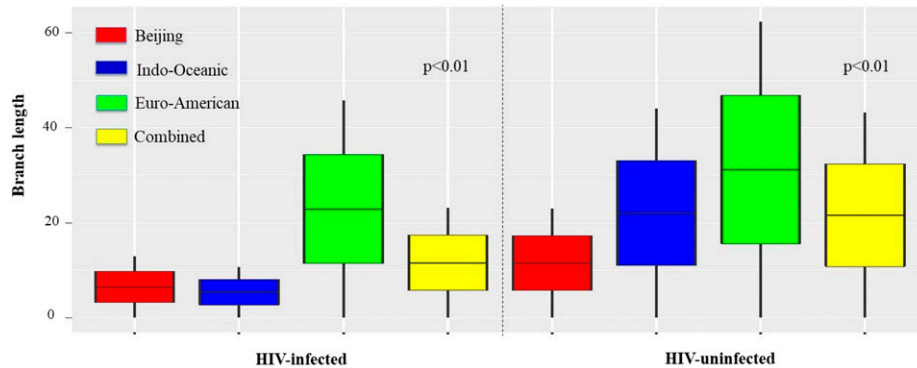


FIGURE 4. Comparison of branch length by *Mycobacterium tuberculosis* strain lineage in HIV-infected and uninfected patients. HIV = human immunodeficiency virus. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

age-related effect with shorter branch lengths noted in strains isolated from HIV-infected patients younger than 45 years compared with those  $\geq 45$  years of age ( $P < 0.01$ ).

## DISCUSSION

This is the first study to assess the *M. tuberculosis* transmission dynamics among HIV-infected patients in Vietnam.<sup>8,16</sup> We did not observe large strain clusters, indicative of recent transmission and potential poor infection control practices in health-care centers, as documented in KwaZulu-Natal, South Africa.<sup>26</sup> Being able to compare strains from HIV-infected patients with those in the general community provided an unprecedented opportunity to map the dynamic exchange of *M. tuberculosis* strains between HIV-infected and uninfected individuals in the same population. The fact that strains from HIV-infected patients were interspersed with community strains indicates that most HIV-infected patients acquired *M. tuberculosis* infection in the general community, with limited person-to-person transmission among HIV-infected patients. Although the absence of big clonal outbreaks among TB/HIV coinfecting patients is comforting from an infection control perspective, more effort is still justified to limit the TB exposure of HIV-infected patients within the community.

Given the limited resolution of MIRU-24 and spoligotyping to differentiate Beijing lineage clusters, WGS allowed more accurate cluster characterization.<sup>15,27</sup> Whole genome sequencing revealed variation between isolates that were indistinguishable by MIRU-24 and illuminated new clusters that were not detected by MIRU-24. The observation that some strains with nonidentical MIRU-24 profiles, especially those with multiple loci differences, were recognized as part of a WGS cluster seems counterintuitive, given that MIRU-24 has reduced resolution compared with WGS. This might be explained by the fact that repetitive regions of the *M. tuberculosis* genome, including the variable number tandem repeats used for MIRU typing, are excluded from genomic comparisons and analysis (e.g., regions annotated as PE/PPE/PGRS and *cysA* genes, insertion sequences, transposases, and prophage components)<sup>15</sup> and, therefore, MIRU-24 may capture some genome variability that is not detected by current WGS analysis methods. Compared with MIRU-24, WGS provided the greatest resolution improvement in Beijing lineage strains, which display the least MIRU-24 allelic diversity.<sup>15</sup> In our study, WGS also demonstrated added value in

the evaluation of Indo-Oceanic and Euro-American lineage strains, with the identification of additional clusters not recognized by MIRU-24.

The significantly shorter phylogenetic tree branch lengths observed among TB/HIV coinfecting patients, compared with strains from HIV-uninfected patients, suggest more recent transmission with a shorter time between infection and disease progression. As similar observations of Holt and others<sup>16</sup> has previously been made comparing Beijing compared with other strain lineages. However, the use of branch lengths as a measure of recency of infection can be confounded by within host dynamics,<sup>28</sup> as well as an age cohort effect among TB/HIV coinfecting patients who were generally younger in our study. Most TB/HIV coinfecting patients were less than 45 years of age, whereas HIV-uninfected TB patients in Vietnam are typically older.<sup>29</sup>

Although we found no evidence of a dominant drug-resistant clone, as reported recently in Papua New Guinea,<sup>30</sup> the high rate of drug resistance within identified strain clusters is alarming because it confirms frequent transmission of drug-resistant strains within the community. The increased clustering of drug-resistant strains that we observed might have been biased by the fact that we did not sequence all drug-susceptible isolates. However, our findings are consistent with household contact surveys that suggested increased TB transmission within MDR-TB-affected households in Vietnam.<sup>31</sup> Overall, the rate of drug-resistant disease among TB/HIV coinfecting patients was high and the fact that streptomycin resistance was most common aligns with previous findings from Vietnam<sup>32,33</sup> and Mongolia.<sup>34</sup> In Vietnam, this can be partially explained by the perception that there is a preference for intramuscular injections and the fact that streptomycin was used during the intensive phase of standard first-line treatment in preference to ethambutol until 2016.<sup>32,35</sup>

Our findings also extended the catalog of significant genomic changes that can be used to infer drug resistance in strains circulating in Southeast Asia. As in other settings, mutations at codons 43 and 88 of the *rpsL* gene were most frequent and associated with high-level streptomycin resistance.<sup>36,37</sup> Mutations in *gidB*, which has been associated with a low-level of streptomycin resistance,<sup>36,37</sup> were variable and found in both phenotypically resistant and susceptible strains. Strains harboring *gidB* mutations associated with phenotypic streptomycin resistance, without concurrent *rrs* or *rpsL* mutations, were mostly found in non-Beijing isolates,

similar to observations from Thailand.<sup>37</sup> No *rrs* mutation associated with streptomycin resistance provided cross-resistance with second-line injectable drugs such as amikacin or kanamycin.<sup>38</sup> The small number of discordant phenotypic and genotypic streptomycin resistance profiles may be due to imperfect phenotypic results or potential culture contamination.<sup>39,40</sup>

Whole genome sequencing identified three additional MDR-TB cases that were not recognized by routine phenotypic methods,<sup>12</sup> which fuels debate about the most appropriate critical concentrations and reference standards for MDR-TB determination. It has been noted before that strains harboring “high-confidence” drug resistance mutations may sometimes be missed by phenotypic DST.<sup>41</sup> In some discrepant cases, the critical concentrations of antibiotic used for phenotypic DST may be inadequate to detect lower levels of resistance, which is relevant in *rpoB* mutations associated with low-level rifampicin resistance.<sup>42</sup> However, genotypic results may also be affected by incomplete catalogs of resistance associated mutations and/or potential misclassification of lineage-specific polymorphisms as resistance mutations.<sup>43</sup> S450L has been the most common mutation associated with rifampicin resistance,<sup>44</sup> and this was the case among HIV-infected and HIV-uninfected patients in Vietnam. Most *rpoB* mutations would have been detected by Xpert MTB/RIF<sup>®</sup> which is widely available and used in Vietnam (23), but the I491F mutation detected in two unrelated strains would have been missed, even by the new Ultra version.<sup>45</sup> This mutation is outside the 81-bp RRDR and has been recognized as problematic by the CRyPTIC consortium,<sup>46</sup> with potential for diagnostic selection observed in Swaziland.<sup>47</sup>

Culture-based DST for pyrazinamide and ethambutol remains challenging.<sup>48</sup> We identified resistance-conferring mutations to ethambutol by WGS, but many of these strains appeared to be phenotypically susceptible. It has been suggested that the suboptimal sensitivity of phenotypic ethambutol resistance testing may be due to the presence of bacterial subpopulations<sup>49,50</sup> or drug concentrations that are too high to detect all clinically relevant resistance.<sup>51</sup> We also found three common *gyrA* mutations that have been associated with high-level resistance to fluoroquinolones<sup>52,53</sup> in isolates without a MDR-TB phenotype. This may reflect a risk of using fluoroquinolone treatment of community-acquired pneumonia or other common infections, especially in HIV-infected patients at high risk of TB.<sup>54,55</sup>

Several study limitations have to be acknowledged. First, the fact that sample collection was noncontinuous, although consecutive samples were collected at times (2009–2010 and then again in 2014) when no other studies were in progress. However, we believe that our findings remain informative, given that consecutive sample collection was unbiased and the sampling periods were broadly comparable to the study of HIV-uninfected patients performed by Holt et al.<sup>17</sup> We cannot exclude the possibility that some of the TB cases included in Holt’s study may have been included in our study as well, but, given good HIV and TB record keeping in Ho Chi Minh City, we believe the chances for this are small and unlikely to have had any significant influence on our results. Second, we have already commented on the predominance of young males in our study, which reflects the fact that the HIV epidemic in Vietnam primarily affects injecting drug users and men who have sex with men.<sup>56</sup> This limits the generalizability of our findings to

settings with different demographics, such as sub-Saharan Africa, where HIV transmission is mostly heterosexual and affects equal numbers of men and women.<sup>57</sup>

In conclusion, drug resistance in *M. tuberculosis* in TB-HIV coinfecting patients was associated predominantly with Beijing and Indo-Oceanic lineage strains and the broad spectrum of resistance-conferring mutations. *Mycobacterium tuberculosis* transmission among people living with HIV in Ho Chi Minh City appears to be limited, but our findings suggest extensive *M. tuberculosis* transmission between HIV-infected and uninfected individuals within the community, with high rates of transmitted drug-resistant TB. These findings highlight the added value of genome sequencing-based analysis in tracking transmission pathways and encourage further efforts to reduce TB transmission within the general community, with specific emphasis on the optimal use of preventive therapy in HIV-infected individuals.

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