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Epidemiology

Tuberculosis risk factors and *Mycobacterium tuberculosis* transmission among HIV-infected patients in Vietnam

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1. Background

Effective tuberculosis (TB) control poses a major public health challenge in Vietnam. Vietnam ranked 16th among high-burden TB countries and 13th among high-burden multi-drug resistant (MDR) TB countries in 2016, with MDR-TB reported in 4.1% of new and 26% of retreatment cases [1]. The emergence of MDR-TB threatens recent progress in TB control, especially in settings where co-infection with human immunodeficiency virus (HIV) complicates the situation even further [2]. Patients with TB/HIV co-infection experience increased mortality if initiation of antiretroviral treatment (ART) is delayed or first-line TB treatment is suboptimal in the absence of drug susceptibility testing (DST) [3,4].

A recent retrospective study conducted in Ho Chi Minh city, Vietnam, suggested that TB/HIV co-infection is associated with relatively high rates of drug resistance in *Mycobacterium tuberculosis* [5]. This is concerning, since the risk of nosocomial TB transmission among people living with HIV, including highly drug resistant strains, is well documented [6]. Whole genome sequencing (WGS) offers the opportunity of assessing drug resistant mutations and tracking transmission with unprecedented strain resolution. This is especially important in highly clonal Beijing lineage strains of *M. tuberculosis* which predominate in Vietnam but are poorly differentiated by other methods of subtyping [7]. Findings from the study in Ho Chi Minh city suggested occasional TB transmission among people living with HIV, with most transmission occurring in the general community [3]. However, the study was limited by its retrospective nature, potentially biased non-consecutive sampling and limited geographic representation. To address these limitations, we conducted a prospective study of consecutive TB samples collected in Hanoi and Ho Chi Minh city. Prospective data collection also allowed more detailed assessment of risk factors associated with TB and HIV infection and the possibility to distinguish relapse from re-infection among patients with TB recurrence.

2. Methods

We prospectively recruited new pulmonary TB cases with their diagnosis confirmed by sputum microscopy for acid fast bacilli (AFB) or GeneXpert MTB/RIF® (Xpert, Cepheid, USA), in Ho Chi Minh city (Pham Ngoc Thach Hospital) and Hanoi (Hanoi Lung Hospital and 09 HIV/AIDS

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Hospital). All patients diagnosed with TB/HIV co-infection between 1 January 2015 and 31 December 2017 were enrolled in the study. Patients who were unable or unwilling to provide written informed consent, were already established on TB treatment (> 30 days), were not local residents or considered to be at high risk of death (which would preclude follow-up for a minimum period of 12 months) or tested positive for rifampicin resistance on Xpert were excluded. Demographic data, risk factors for TB and HIV infection, treatment and outcome data were collected using a detailed questionnaire (on-line Supplement). Patients were managed by the Vietnam National Tuberculosis Program (NTP) and followed for a minimum of 12 months (range 1–3 years) for treatment completion and/or TB recurrence.

2.1. Study setting and specimen collection

Pham Ngoc Thach Hospital is the main HIV referral centre and manages the largest number of TB/HIV co-infected patients in southern Vietnam. In northern Vietnam, Hanoi Lung Hospital is the main referral centre for TB and 09 Hospital - for HIV/AIDS patients. Between them they manage the largest number of TB/HIV co-infected patients in northern Vietnam. In each patient, sputum specimens were collected for smear microscopy and/or Xpert using standard methods. In patients with smear or Xpert positive sputum, mycobacterial cultures were performed using either solid (Lowenstein–Jensen, bioMérieux SA, Marcy-l'Étoile, France) or liquid (BACTEC or MGIT 960; Becton Dickinson & Co., Franklin Lakes, NJ) culture media. *M. tuberculosis* isolates were identified using observation of typical colonies and the niacin test [8].

2.2. DNA extraction, whole genome sequencing and bioinformatics analysis

Genomic DNA was extracted from positive cultures as per standard protocol [9] and libraries constructed using the Nextera XT DNA preparation kit (Illumina, San Diego, California). Sequencing was performed on the NextSeq 500 (Illumina, San Diego, California) platform and reads mapped to H37Rv as the reference genome using the RedDog pipeline (https://github.com/katholt/RedDog) [10]. An initial phylogeny was inferred using FastTree v2.1.8 [11]. Careful checks for low frequency variants were performed and variant calling was done using Snippy version 3.1 (https://github.com/tseemann/snippy). For consistency with the previous retrospective study, genomes with ≤10 SNP differences (calculated as the number of SNP differences between individual members of the cluster compared to the ancestral node) were classified as a WGS cluster [7,12]. Strain sequences from HIV-uninfected TB patients in Ho Chi Minh city, diagnosed between 2015 and 2017, were used in comparative genomics analyses [10]. Raw short read sequence data were submitted to the Sequence Read Archive at the National Centre for Biotechnology Information (NCBI) (BioProjects PRJNA506272).

Maximum likelihood phylogenetic trees with 1000 bootstraps were constructed using RAxML (version 8.2) and FastTree (version 2.1.8) [11] using the Generalised Time Reversible (GTR) model of nucleotide substitution and a Gamma model of rate heterogeneity to analyze a concatenated alignment of SNP alleles. Visualization of trees was done through Microreact (www.microreact.org) and Figtree (version 1.4.3). Ancestral sequence reconstruction was performed using FastML (version 3.1). All statistical analysis was performed using SPSS v.20.0 (IBM Corp., Armonk, NY, USA). Difference with $p < 0.05$ was considered to be statistically significant.

2.3. Ethics approval

The study was approved by the Ethics Committee of National Institute of Hygiene and Epidemiology, Hanoi (IRB-VN01059-13/2015, 13 July 2015) and Pham Ngoc Thach Hospital Ethics Review Committee, Ho Chi Minh city (188/PNT-NCKH, 22 June 2015).

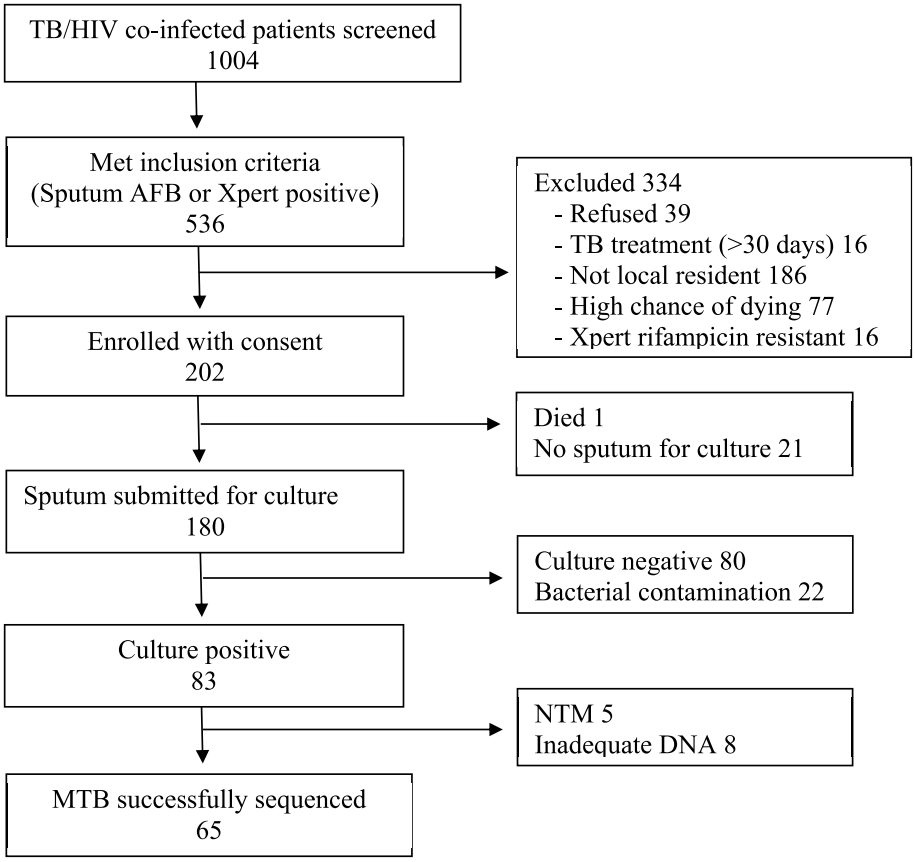


Fig. 1. Flow diagram of TB/HIV co-infected patients enrolled.

3. Results

A total of 1004 patients with TB/HIV co-infection were identified during the study period; 536 (53.4%) were new pulmonary TB cases. Given that recruitment mainly occurred at tertiary referral hospitals, many TB cases were excluded as non-local residents or for being established on TB treatment by the time of recruitment (186; 18.5%), while 77 (7.7%) were considered to be at high risk of dying. Fig. 1 presents a detailed overview of patients enrolled in the study. Of those tested with Xpert 16/391 (4.1%) were considered rifampicin resistant and excluded from the study. Table 1 describes the demographic profile of the 202 TB/HIV co-infected patients ultimately enrolled; *M. tuberculosis* was cultured from 83 patients (41.1%) and successfully sequenced in 65 cases (78.3% of all cultured cases). The majority of patients (78.7%) were young males (median age 35 years; range 18–62 years). Few (33.2%) reached an education level beyond grade 10 and the reported median monthly income in the study cohort was US $175.

Table 2 provides a summary of TB and HIV risk factors recorded. Cigarette smoking was common (57.9%), especially among men (111/159; 69.8%). The average duration of cigarette smoking was 15 years, among those who answered the question (84.6%). Around a third (33.7%) of patients reported excessive alcohol intake (> 3 alcohol drinks > 3x/week), and a similar percentage (34.2%) admitted illicit drug use; 15/69 (23.1%) indicating illicit drug use in the previous month. Intravenous drug use was a major risk factor for HIV acquisition, especially in Hanoi (26/46; 56.5%), while unsafe sex was the major risk factor identified in Ho Chi Minh city (65/156; 41.7%).

Table 1
Demographic profile of TB/HIV co-infected patients in Hanoi and Ho Chi Minh City, Vietnam.

Characteristics	Total N = 202 n (%)	Hanoi N = 46 n (%)	HCMC N = 152 n (%)
Gender			
Male	159 (78.7)	37 (80.4)	122 (78.2)
Age group			
< 18	1 (0.5)	1 (2.2)	0
18–24	11 (5.5)	1 (2.2)	10 (6.4)
25–34	74 (36.6)	12 (26.1)	62 (39.7)
35–44	84 (41.6)	21 (45.6)	63 (40.4)
≥45	32 (15.8)	11 (23.9)	21 (13.5)
Education status[a]			
No primary school	6 (3.0)	0 (0)	6 (3.9)
Primary school	49 (24.3)	9 (19.6)	40 (25.6)
Secondary school	78 (38.6)	21 (45.7)	57 (36.5)
High school	46 (22.7)	14 (30.4)	32 (20.5)
Graduate and post graduate	23 (11.4)	2 (4.3)	21 (13.5)
Work status			
Unemployed	56 (27.7)	14 (30.4)	42 (26.9)
Self-employed	71 (35.1)	25 (54.4)	46 (29.5)
Government officer	3 (1.5)	0	3 (1.9)
Company worker	28 (13.9)	5 (10.9)	23 (14.7)
Student	2 (1.0)	0	2 (1.3)
Other	42 (20.8)	2 (4.3)	40 (25.7)
Economic status			
Poor	32 (15.8)	7 (15.2)	25 (16.0)
Borderline poor	37 (18.3)	6 (13.0)	31 (19.9)
Not poor	86 (42.6)	12 (26.1)	74 (47.4)
Unknown	47 (23.3)	21 (45.7)	26 (16.7)
Partner status			
Single	84 (41.6)	21 (45.6)	63 (40.4)
Permanent partner	87 (43.1)	20 (43.4)	67 (42.9)
Separated/Widowed	28 (13.8)	4 (8.7)	24 (15.4)
Unknown	3 (1.5)	1 (2.3)	2 (1.3)

HCMC – Ho Chi Minh City.

[a] Highest education level achieved – Primary (grade 1–5), Secondary (grade 6–9), High school (grade 10–12); **As defined by the Vietnam government multi-dimensional poverty standard for 2011–2015 (Decision No. 09/2011/QD-TTg).

The most likely TB exposure in the last 2 years was reported to have occurred at home, but nearly 9/46 (19.8%) patients in Hanoi indicated time spent in a drug rehabilitation centre. Very few cases had other "high risk" institutional exposures (i.e. military service 1.0%; time in prison 3.5%). Since only new TB cases were enrolled, very few patients reported previous TB treatment (2.0%). Importantly, 175 (86.6%) TB/HIV co-infected patients never received TB preventive therapy and many (39.1%) were diagnosed with HIV during this TB episode. Among those with previously known HIV infection, the average number of years since HIV diagnosis was 4.5 years and the average duration of those on anti-retroviral therapy (ART) (47.0%) was 2.1 years. Among the 114 patients on ART, treatment adherence was considered acceptable in the majority (80.7%), with better reported adherence in Ho Chi Minh city (84/92; 91.3%) than in Hanoi (8/22; 36.3%, $p < 0.05$).

Cough, fever and weight loss were the most common symptoms reported of TB/HIV co-infected patients, with night sweats in a minority (Table 3). The chest X-ray was highly suggestive of TB in 93.0% of cases. The majority of cases (96.0%) were sputum smear positive (46; 22.8% were 2 + or 3 + positive) and 92.6% were Xpert positive (including one case reported as rifampicin resistant); frequently performed after treatment initiation. Only 34.1% of patients were hospitalized for TB treatment initiation. The most commonly used TB treatment

Table 2
Risk factors for TB and HIV among TB/HIV co-infected patients in Hanoi and Ho Chi Minh City, Vietnam.

Characteristics	Total N = 202 n (%)	Hanoi N = 46 n (%)	HCMC N = 156 n (%)
Cigarette smoking	117 (57.9)	27 (58.7)	90 (57.7)
Excessive alcohol intake[a]	68 (33.7)	11 (23.9)	57 (36.5)
Illicit drug use			
Previous drug use	69 (34.2)	30 (65.2)	39 (25.0)
Drug use in past month (n = 69)	15 (23.1)	9 (34.6)	6 (15.4)
Methadone treatment (n = 69)	9 (4.5)	2 (4.4)	7 (4.5)
Institutional exposure (last 2 years)			
Military	2 (1.0)	1 (2.2)	1 (0.6)
Drug treatment centre	13 (6.4)	9 (19.8)	4 (2.6)
Prison	7 (3.5)	4 (8.7)	3 (1.9)
Co-morbidity (doctor diagnosed)			
Liver disease	26 (12.9)	9 (19.6)	17 (10.9)
Diabetes mellitus	3 (1.5)	0 (0)	3 (1.9)
BCG vaccination scar	100 (49.5)	11 (23.9)	89 (57.1)
Close TB contact			
Close TB contact ever	41 (20.3)	9 (19.6)	32 (20.5)
During the last 2 years	21 (10.4)	5 (10.9)	16 (10.3)
TB preventive therapy			
Never	175 (86.6)	32 (69.6)	143 (91.7)
Do not know	12 (5.9)	8 (17.4)	4 (2.6)
Previous TB treatment[b]			
No	190 (94.1)	39 (84.8)	151 (96.8)
Yes	4 (2.0)	2 (4.4)	2 (1.3)
Uncertain	6 (3.0)	4 (8.7)	2 (1.3)
Likely source of HIV infection			
Injection drug use	57 (28.4)	26 (56.5)	31 (19.9)
Unsafe sex	75 (37.1)	10 (22.2)	65 (41.7)
Do not know	70 (34.7)	9 (19.6)	60 (38.5)
Current Antiretroviral treatment (ART)			
Yes	114 (56.4)	28 (60.9)	86 (55.1)
No	79 (39.1)	17 (37.0)	62 (39.7)
Not available	9 (4.5)	1 (2.2)	8 (5.2)
[c]Initiated during this TB episode	100 (49.5)	24 (52.2)	76 (48.7)
ART adherence (doctor impression; n = 114)			
Poor (≤79% of doses)	22 (19.3)	14 (63.7)	8 (8.7)
Acceptable (≥80% of doses)	92 (80.7)	8 (36.3)	84 (91.3)

TB – tuberculosis; HCMC – Ho Chi Minh City; BCG: bacille Calmette-Guérin vaccine.

[a] Excessive alcohol intake - > 3 alcohol drinks > 3 times/week.

[b] Study criteria only included new TB cases.

[c] Initiated during this TB episode – subgroup of those on current ART.

Table 3
Clinical presentation and management of TB/HIV co-infected patients in Hanoi and Ho Chi Minh City, Vietnam.

Characteristics	Total N = 202 n (%)	Hanoi N = 46 n (%)	HCMC N = 156 n (%)
TB Diagnosis			
Symptoms at presentation			
Cough	166 (82.2)	38 (82.6)	128 (82.1)
Fever	157 (77.7)	38 (82.6)	119 (76.3)
Weight loss	152 (75.2)	42 (91.3)	110 (70.5)
Night sweats	91 (45.0)	28 (60.9)	63 (40.4)
Suggestive Chest X-Ray	188 (93.0)	35 (76.0)	153 (98.1)
Sputum AFB positive	194 (96.0)	39 (84.8)	155 (99.4)
GeneXpert MTB/RIF®			
MTB positive	187 (92.6)	46 (100)	141 (90.4)
Rifampicin resistant[a]	1 (0.5)	1 (2.2)	0
TB treatment			
Type of treatment			
Out patient	121 (59.9)	19 (41.3)	102 (65.4)
In patient	12 (5.9)	10 (21.7)	2 (1.3)
Both	57 (28.2)	11 (23.9)	46 (29.5)
Unknown	12 (5.9)	6 (13.0)	6 (3.9)
TB treatment regimen			
2RHZE/4RHE	191 (94.6)	43 (93.5)	148 (94.9)
2SHRZE/6RHZE	2 (1.0)	0	2 (1.3)
2SRHZE/1RHZE/5RHZ	1 (0.5)	1 (2.2)	0
3RHZE/5RHE	1 (0.5)	1 (2.2)	0
6HZE	2 (1.0)	0	2 (1.3)
Unknown	5 (2.5)	1 (2.2)	4 (2.6)
HIV treatment			
CD4 count – median (interquartile range)	78.5 (2–663)	80.5 (16–652)	66.5 (2–654)
Current antiretroviral treatment (ART)	114 (56.4)	28 (60.9)	86 (55.1)
ART initiated during this TB episode	100 (49.5)	24 (52.2)	76 (48.7)
ART regimen (n = 100)			
At least 3 drugs	49 (49.0)	15 (62.5)	34 (44.7)
< 3 drugs (sub-optimal)	10 (10.0)	0 (0)	10 (13.2)
Unknown	41 (41.0)	9 (37.5)	32 (42.1)

TB – tuberculosis; MTB – *Mycobacterium tuberculosis*; HCMC – Ho Chi Minh City.
[a] In total 17/391 (4.3/%) Xpert MTB/RIF® positive cases had genotypic rifampicin resistance; 16 were excluded from genotyping and follow-up as this was an exclusion criterion, but one was incorrectly enrolled.

regimen was 2 months rifampicin, isoniazid, pyrazinamide, ethambutol, followed by 4 months of rifampicin, isoniazid, ethambutol (2RHZE/4RHE), used in 94.6% of patients. A variety of non-standard regimens were used in a small minority of patients. The mean CD4 T-cell count at first examination was 160.9 cells/mm^3 (range 2–999); indicative of severe immune suppression at the time of diagnosis. Nearly half of the patients (49.5%) on ART had their therapy initiated during this TB episode; in 10 cases the treatment consisted of sub-optimal regimens using < 3 ART drugs.

Beijing lineage of *M. tuberculosis* was the predominant phylogenetic lineage in our study (44/65; 67.7% of sequenced strains), followed by Indo-Oceanic (24.6%) and Euro-American lineage strains (7.7%). The analysis of sequenced genomes identified 3 clusters, all composed of Beijing lineage strains. One 2 member and one 4-member cluster involved Hanoi patients enrolled at 09 Hospital and another 4-member cluster 2 cases from Hanoi and 2 from Ho Chi Minh city (Fig. 2). Two of the clusters were not suggestive of recent transmission, differing by > 5 SNPs and without any identified epidemiological links. Two strains within a 2-member cluster were identical and both from Hanoi. However, these patients were from different areas of residency, and their specimens were processed in different labs. No evidence of direct contact or laboratory contamination could be established. Fig. 3 reflects a combined analysis of the Ho Chi Minh city strains sequenced during this prospective study (2015–2017) and a previous retrospective study (2009–2014) [13], compared to community strains (2008–2011) sequenced by Holt et al. [10]. The combined analysis identified no large strain clusters among TB/HIV co-infected patients, suggestive of potential nosocomial transmission.

The drug resistance conferring mutations identified in relevant *M. tuberculosis* strain lineages are presented in Fig. 4. Despite the fact that Xpert detected rifampicin resistance was a criterion for study exclusion, 6/65 (9.2%) sequenced isolates were genotypically identified as MDR. The most common *rpoB* mutation responsible for rifampicin resistance was S450L (4/8; 50%), which is inside the rifampicin resistance determining region (RRDR) targeted by Xpert. However, one rifampicin monoresistant and one MDR strain, both from the Beijing lineage, carried mutations (L452P and I491F, respectively) located outside the RRDR. For isoniazid, the *kat*G S315T mutation was responsible for 60.0% (18/30) of the resistance mutations observed, followed by C-15T in the *fab*G1-*inh*A regulatory region (6/30; 20.0%). Streptomycin resistance was mainly mediated by a *rpsL* K43R mutation (11/19; 57.9%) found in Beijing lineage strains (10/11; 90.9%). Mutations conferring resistance to ethambuthol, pyrazinamide and quinolones were infrequent.

4. Discussion

This is the first study to assess TB risk factors among people living with HIV in Vietnam. Studies from around the world [14–17] suggested a range of risk factors, including: male gender, previous TB disease, low body mass index, low CD4 cell count, advanced HIV disease stage, urban setting and co-infection with hepatitis C, which is often a marker of intravenous drug use [18]. Our study was congruent with previous reports, indicating a high risk among young men with advanced HIV disease and delayed ART initiation in Vietnam. The significance of other risk factors, such as a history of incarceration, cigarette smoking and excessive alcohol intake were difficult to establish, but is well documented in other studies [19–21].

The poor uptake of TB preventive therapy, even among patients known to be HIV infected prior to their TB diagnosis, is an important

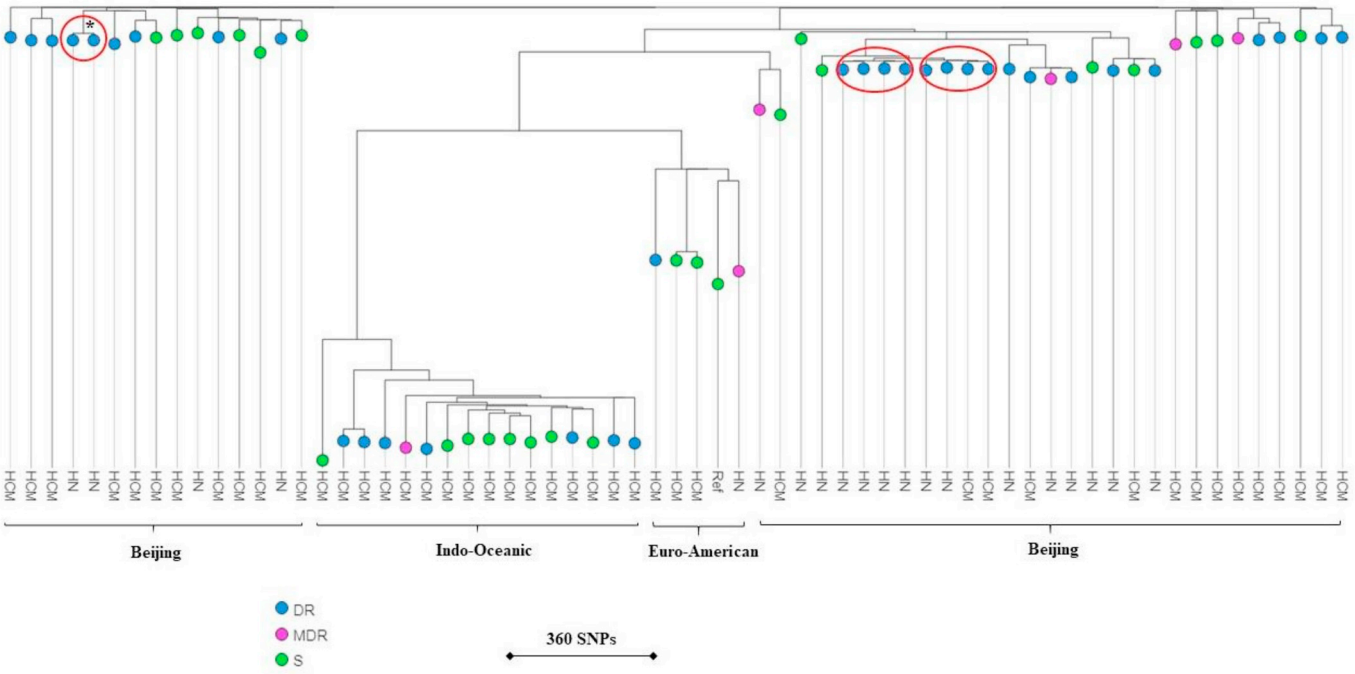


Fig. 2. Phylogenetic tree of *M. tuberculosis* strains isolated from TB/HIV co-infected patients in Vietnam.

reversible risk factor. The protective effect of isoniazid preventive therapy in people living with HIV is well established [22,23]. The World Health Organization (WHO) recommends the three I's approach, which includes (i) intensified TB case-finding, (ii) isoniazid preventive therapy, and (iii) infection control to prevent TB in people living with HIV [24]. Another important TB prevention component is early ART initiation, which is part of the ambitious 90/90/90 strategy for HIV elimination [25]. Universal early ART initiation provides strong synergy with isoniazid preventive therapy [24] to reduce TB incidence and deaths among people living with HIV. Observational studies from South Africa and Brazil [22,26] indicated that combined isoniazid preventive therapy and ART was superior to ART or isoniazid preventive therapy alone in reducing TB incidence among adults living with HIV. However, the high rate of isoniazid resistance observed in the study cohort is a major concern, since it reduces the likely efficacy of isoniazid preventive therapy and acts as an important gateway for the emergence of MDR strains [27].

Delayed initiation of ART was common in our study, as evidenced by the high degree of immune compromise observed and the fact HIV infection was often only detected after a TB diagnosis was made. Early ART is the most effective measure to reduce TB susceptibility among people living with HIV [15,28,29]. TB incidence may transiently increase in the first year after ART initiation [30,31], but this is only observed if patients are already immune compromised when treatment is initiated and immune reconstitution unmasks undetected pre-existing TB [32,33]. Furthermore, intravenous drug use was high among young men, especially in Hanoi. Drug use among women may be more difficult to ascertain, given potentially increased stigma, but the observations are generally consistent with physician experience [34] and the over-representation of young men (22–35 years old) in drug rehabilitation centers in Vietnam [35,36]. Early diagnosis and treatment of HIV in all high risk groups, including intravenous drug users, should be a key component of national strategies to control HIV and the TB/HIV co-circulation in Vietnam [28,37].

Beijing lineage was the dominant strain lineage observed, which is similar to observations in the general community in Vietnam [10,38,39]. This phylogenetic lineage has also been associated with HIV infection status in South Africa [40]. All clustered strains, which included drug resistant strains, were Beijing lineage strains and its association with drug resistance and increased transmission, especially among younger populations, have been demonstrated in other studies [10,41–43]. There was no evidence suggestive of nosocomial transmission within hospitals or district tuberculosis units, since all clustered strains were from different locations; including a single identical pair in whom no direct contact or laboratory contamination event could be established. However, there has been concern that poor infection control practices in health facilities in Vietnam may facilitate nosocomial TB transmission [44]. In Ho Chi Minh city, the combined analysis of our current dataset, a previous retrospective study [5] and recent findings in non-HIV infected patients [10] demonstrated that strains from TB/HIV co-infected patients were intermingled with strains circulating in the general community [45,46]. No significant size clusters suggestive of nosocomial transmission among hospitalized TB/HIV co-infected patients were observed, as documented during the XDR-TB outbreak in Kwazulu Natal, South Africa [47].

As expected, Xpert missed the two resistance conferring mutations in *rpoB* gene located outside the RRDR [48,49]; one of which (*rpoB* I491F) has demonstrated clonal expansion under Xpert diagnostic selection pressure in Swaziland [50] and are likely to be missed by Xpert MTB/RIF Ultra® as well [51,52]. Interestingly, 6 cases with RRDR mutations were also undetected. This might be explained by wrong interpretation of the test result or lower sensitivity of Xpert for *M. tuberculosis* detection in patients with a low sputum bacterial load following TB treatment initiation [53,54]. Importantly, isoniazid mono-resistance was prevalent in this cohort, but this might be influenced by the exclusion of cases with Xpert detected rifampicin resistance. However, it is important to consider that isoniazid resistance is the usual gateway to MDR-TB. Genotypic findings support previous observations that resistance conferring mutations *rpoB* S450L, *kat*G S315T, *fab*G1 C-15T and *rpsL* K43R have been common substitutions associated with resistance to first-line TB drugs in Vietnam [13,55,56]. Quinolone resistance was less common, but found in most sites and lineages, which is a concern given importance of quinolones in MDR treatment regimens [57].

Study limitations include the exclusion of cases with Xpert positive rifampicin resistance, which limits our ability to comment on drug

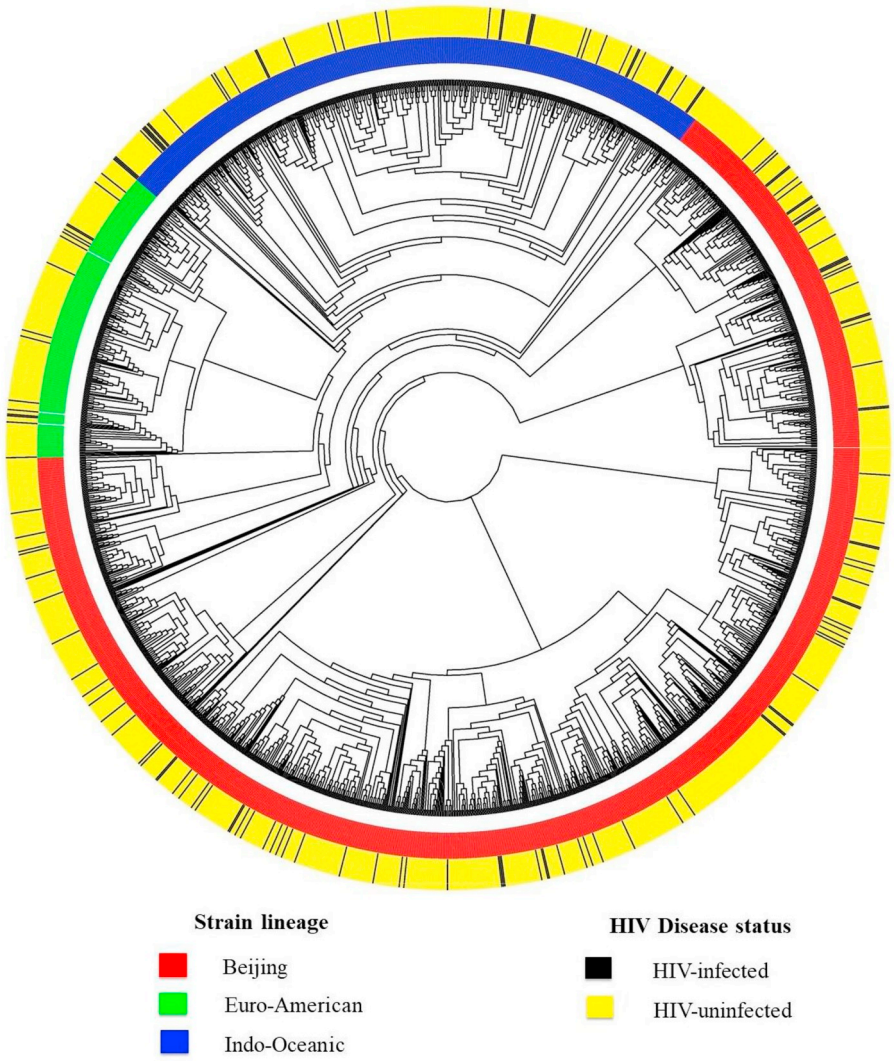


Fig. 3. *M. tuberculosis* strains identified from TB/HIV co-infected patients in Ho Chi Minh City from prospective and retrospective studies, compared to community strains*.

resistance patterns. Since no phenotypic DST was performed, discrepancies between phenotypic and genotypic DST profiles were not considered. The low culture yield observed probably reflects the fact that many patients were recruited into the study after initiation of TB treatment at the referring facility. The cluster definition used (< 10 SNPs) may be too sensitive for a high incidence setting, as suggested by the fact clustered cases were referred from different District Tuberculosis Units (DTUs) without any identifiable epidemiological links. Given the high representation of injecting drug users, men who have sex with men and female sex workers [58–61] among people living with HIV in Vietnam, the generalizability of our findings to settings with different epidemiological profiles, like sub-Saharan Africa [62], maybe limited. Lastly, we were unable to comment on relapse versus reinfection, due to low rates of TB recurrence during the study period.

5. Conclusion

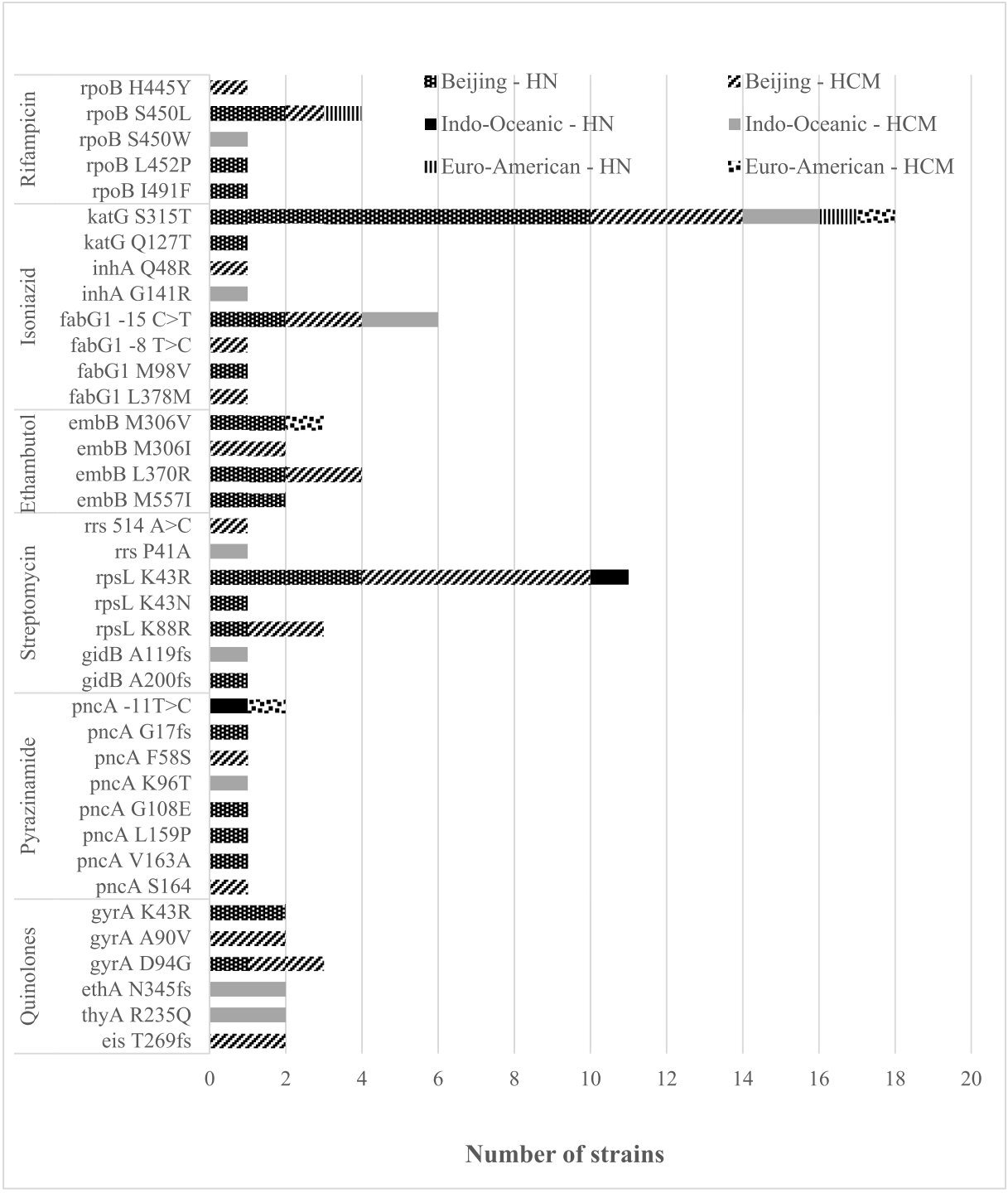
Young men with a history of intravenous drug use were at high risk of TB/HIV co-infection in Vietnam, especially in Hanoi. Poor uptake of TB preventive therapy and delayed ART initiation, even among those known to be HIV infected, represent important modifiable TB risk factors that should be addressed. There was limited evidence suggestive of nosocomial TB transmission among people living with HIV. TB disease in HIV-infected patients mainly resulted from infection acquired in the general community.

Potential conflicts of interest

The authors declare no conflict of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The study is in partial fulfilment of Trinh Quynh Mai's PhD thesis.

Author contributions

Trinh QM, Martinez E, Van Anh NT, Hien NT, Nhung NV, Marais BJ and Sintchenko V conceptualised the study. Trinh QM, Van Anh NT, Hien NT, Lan NH, Giang DC, Hang PT, Thuong PH, Huan HV, Hoang NP, Hoa NB acquired data. Trinh QM, Martinez E analyzed the data. All



TB – tuberculosis; HIV – human immunodeficiency virus;

Location: HN - Hanoi; HCM - Ho Chi Minh City.

Fig. 4. Drug resistance mutations identified in different *M. tuberculosis* strain lineages from TB/HIV co-infected patients in Hanoi and Ho Chi Minh City, Vietnam.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2019.02.001.

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