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Sublineages of *Mycobacterium tuberculosis* Beijing genotype strains and unfavorable outcomes of anti-tuberculosis treatment

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SUMMARY

The influence of *Mycobacterium tuberculosis* (MTB) lineages/sublineages on unfavorable tuberculosis (TB) treatment outcomes is poorly understood. We investigated the effects of Beijing genotype sublineages and other factors contributing to treatment outcome. Patients newly diagnosed with sputum smear-positive and culture-positive TB in Hanoi, Vietnam, participated in the study. After receiving anti-TB treatment, they were intensively followed up for the next 16 months. MTB isolates collected before treatment were subjected to drug susceptibility testing, and further analyzed to determine MTB (sub) lineages and their clonal similarities. Of 430 patients, 17 had treatment failure and 30 had TB recurrence. Rifampicin resistance was associated with treatment failure {adjusted odds ratio = 6.64 [95% confidence interval (CI), 1.48–29.73]}. The modern Beijing genotype was significantly associated with recurrent TB within 16 months [adjusted hazard ratio = 3.29 (95% CI, 1.17–9.27)], particularly after adjustment for the relevant antibiotic resistance. Human immunodeficiency virus coinfection and severity on chest radiographs were not significantly associated with unfavorable outcomes. Our findings provide further understanding of the influence of MTB strains on unfavorable treatment outcomes. Multiple risk factors should be considered for the optimal management of TB.

1. Introduction

Tuberculosis (TB) is a chronic infection that continues to be a major public health problem worldwide, with 8.6 million new cases and 1.3 million deaths in 2012 [1]. Unfavorable treatment outcome, including treatment failure and recurrence, is a major risk factor for drug resistance in TB [2], which increases the TB burden [3]. Recurrence is defined as an active TB episode that reoccurs after initial successful treatment. According to the World Health Organization (WHO) Report 2013 [1], of the 6.1 million cases of TB that were identified, 0.3 million had recurrent episodes (including exogenous reinfection and endogenous reactivation) after being previously cured.

Previous studies have evaluated risk factors for TB recurrence, which include severity of disease indicated on chest radiographs (e.g., the presence of cavitation and the extent of pulmonary involvement) [4,5], drug resistance [6], microbial load at diagnosis, or human immunodeficiency virus (HIV) coinfection [4]. Because recurrence occurs as a result of dynamic interactions between host and pathogen [7], the *Mycobacterium tuberculosis* (MTB) genotype should also be considered a potential risk factor for recurrence. The MTB Beijing genotype strains account for the majority of the East Asian lineage, one of the seven major MTB lineages in the world [8], and are becoming widespread even outside Asia [9]. These strains appear to be associated with an increased risk of TB recurrence, according to several reports [10–13].

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The Beijing genotype strains belong to one of the two major sublineages, ancient (atypical) and modern (typical) types, based on the absence or presence of an IS*6110* insertion in a particular chromosomal position designated as the NTF region of the MTB genome [14]. Molecular epidemiological studies have characterized possible differences in phenotypes between the sublineages: strains of the modern Beijing sublineage are adapted to spread and cause disease more easily than those of the ancient sublineage in different regions of the world [15,16], whereas the ancient Beijing sublineage is often associated with drug resistance, including multidrug resistance (MDR) or extremely drug-resistant TB [17], pyrazinamide (PZA or Z) or rifampicin (RMP or R) resistance [18], and isoniazid (INH or H) or streptomycin (SM or S) resistance [19]. However, associations between Beijing sublineages and unfavorable treatment outcomes have not yet been fully investigated.

Vietnam is a Southeast Asian country stretching over 1,800 km from north to south. It is one of 22 countries with a high TB prevalence worldwide (218 per 100,000 in 2012) [1]. Although the treatment success rate for new cases was reported to be between 85% and 93% from 1995 to 2011 [1], more than 7200 cases were identified as recurrent TB in 2012 [1]. Regional differences in MTB genotypes have been observed. In southern Vietnam, the modern sublineage is reportedly predominant over the ancient sublineage [20]; and the Beijing genotype, as a whole, has been reported as a risk factor for TB recurrence, when compared with non-Beijing genotypes [13]. In Hanoi, in northern Vietnam, the ancient sublineage (37.5%) is more prevalent than the modern sublineage (20.9%) [19]. In the present study, we investigated whether these two Beijing sublineages are similarly associated with treatment failure or recurrence in newly diagnosed active pulmonary TB patients in Hanoi.

2. Materials and methods

2.1. Ethics statement

Written informed consent was obtained from each participant; parents provided written informed consent for minors. This study was approved by the ethical committees of the Ministry of Health of Vietnam, the National Center for Global Health and Medicine, and the Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Japan.

2.2. Study sites, recruitment of patients, and sample collection

Patients were recruited within part of a cohort study reported elsewhere [21]. Residential areas of the patients were divided into three categories based mainly on population density, year of establishment, and urbanization speed: suburban (1500–2500 individuals/km^2), old urban (25,000–26,000 individuals/km^2), and new urban (2800–5300 individuals/km^2) areas [22]. In summary, patients over 16 years old residing in the Hanoi area who suffered from newly diagnosed smear-positive pulmonary TB and agreed to participate in the study were recruited from July 2007 to March 2009. These patients were interviewed by pretrained healthcare staff using a structured questionnaire. Before initiation of treatment, sputum specimens and blood samples were collected. Patients then received the standard 8-month regimen that was commonly administered during the study period in Vietnam: INH, RMP, PZA, and SM or ethambutol (EMB or E) for 2 months followed by INH and EMB for 6 months [2S(E)HRZ/6HE]. Drug susceptibility testing was performed retrospectively on the MTB isolates, which were stored in a freezer before anti-TB treatment began. Thus, susceptibility test results were not available while deciding treatment schedules, and the standard regimen was followed according to the national TB control program guidelines at the time of the study.

During treatment, culture tests were repeated when smear tests were confirmed positive at 2, 5, or 7 months. During the 16-month post-treatment follow-up, sputum smear and culture tests were scheduled at 2, 4, 7, 10, and 16 months for all enrolled cases.

2.3. Identification of MTB, drug susceptibility testing and molecular genotyping

MTB, drug susceptibility, and molecular genotypes were identified as previously reported [19,22]. In short, the niacin test was initially used for MTB identification, and drug susceptibility testing was performed for INH, SM, RMP, and EMB on the basis of the proportional method recommended by WHO. Beijing and non-Beijing strains were distinguished by a single-nucleotide polymorphism (SNP) at position 779,615 [23] and spoligotyping results [24]. Their genotypes were identified using the international MTB database (SpolDB4) [25]. Ancient and modern Beijing genotypes were further distinguished using the polymerase chain reaction method [26]. Variable number of tandem repeat (VNTR) analysis was performed using the international standard 24 mycobacterial interspersed repetitive unit-VNTR system [27] with four additional loci recommended for the Beijing genotype strains [28]. Genetic clustering was defined by a complete match of the VNTR profile of the 28 loci.

2.4. Definitions of treatment failure and TB recurrence

Treatment failure and TB recurrence were defined on the basis of the WHO Global Tuberculosis Report 2013 [1]. In summary, treatment failure was noted when the smear and culture were positive at $\geq$5 months or when the smear was positive but culture was not performed, clinical and/or chest radiography findings indicated failure, and the category switched to the regimen for retreatment. Recurrence was noted when patients were cured after treatment and then suffered a second TB episode. The second episode was confirmed if the sputum culture was positive at the time of recurrence, or if a culture result was not available or was difficult to assess (<5 colonies) [29], but the smear was positive in patients with clinical and/or chest radiographic abnormalities indicating the necessity of retreatment. After the expert committee members' review in Vietnam, the category II regimen (2SHRZE/1HRZE/5$H_3R_3E_3$) for retreatment was started in all patients with treatment failure or TB recurrence.

2.5. Statistical analysis

Logistic regression models were used to investigate factors possibly associated with treatment failure, and adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were calculated. Using a logistic regression model, treatment failure was set as a binominal outcome variable, and the explanatory variables were MTB lineages/sublineages and relevant antibiotic resistance. The log-rank test for equality across strata was also used to investigate the association between MTB lineages and time to recurrence. After testing the proportional hazard assumption, Cox models were used to assess multiple risk factors for recurrence, similar to the logistic regression models. Adjusted hazard ratios (aHRs) and 95% CIs were calculated. Statistical analysis was performed using Stata version 12 (StataCorp, College Station, TX, USA), and P values of <0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the study population

In the present study, 489 patients were diagnosed with bacteriologically confirmed pulmonary TB, of whom 430 completed the directly observed treatment, short-course (DOTS) program at the study sites (Figure 1). Adherence to anti-TB therapy was supervised by the healthcare staff, in cooperation with the patients' family members, under the DOTS strategy of the national TB control program.

Of the 430 patients, 183 (42.6%) were aged <35 years, and 341 (79.3%) were male. HIV coinfection was seen in 22 patients (5.1%), 290 patients (70.2%) had cavities on chest radiographs at diagnosis, and 70 patients (16.9%) had infiltrates spreading to more than half of the lung zones. Among the 413 patients in whom MTB strains were genotyped, Beijing genotype strains were identified in 240 (58.1%) patients, of which 152 belonged to the ancient Beijing sublineages, accounting for 36.8% of all strains tested, and 88 strains belonged to the modern Beijing sublineages, accounting for 21.3% of all the strains tested (Table 1).

Treatment outcomes are illustrated in Figure 1. Treatment failure was noted in 17 (4.0%, 95% CI, 2.3–6.3) of the 430 patients. The remaining 413 patients were considered cured, and 403 of them entered the follow-up period. The median of follow-up time was 484 days (95% CI, 483–487 days) after completion of treatment. Recurrence was observed in 30 patients (7.4%, 95% CI, 5.1–10.5) during this period. Of these, 21 patients (70.0%) exhibited culture-positive results. Culture results were not available or were difficult to assess for the remaining 9 patients (30.0%), but all had smear-positive results with clinical/radiographic changes. All 30 patients with recurrent TB received a retreatment regimen of $2SHRZE/1HRZE/5H_3R_3E_3$. The median time to recurrence was 137 days (95% CI, 110–218 days) after the end of their previous treatment episode.

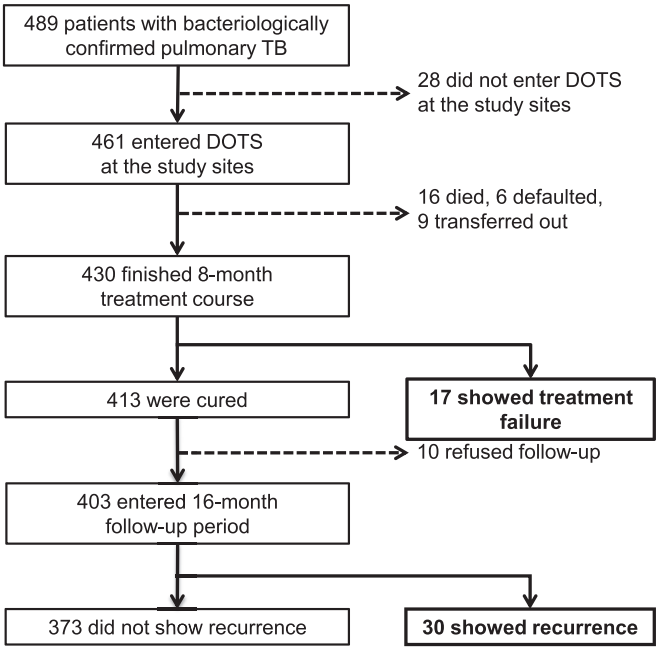

Figure 1. Study flow. TB: tuberculosis; MTB: *Mycobacterium tuberculosis*; NTM: non-tuberculous mycobacterium; DOTS: directly observed treatment, short-course.

Table 1
Characteristics of patients with smear-positive culture-positive pulmonary tuberculosis who finished an 8-month treatment course.

	Total number tested	Number of patients	%
Age (in years)	430		
<25		61	14.2
25–34.9		122	28.4
35–44.9		78	18.1
45–54.9		100	23.3
≥55		69	16.1
Sex	430		
Male		341	79.3
Female		89	20.7
Body mass index	430		
<16		56	13.0
16–18.4		176	40.9
≥18.5		198	46.1
Residential area	430		
Suburban		95	22.1
New urban		199	46.3
Old urban		136	31.6
Smoking habit	429		
Smoker		164	38.2
Ex-smoker		120	28.0
Nonsmoker		145	33.8
HIV status	428		
Positive		22	5.1
Negative		406	94.9
Infiltrate on chest radiograph	414		
≤3 zones		344	83.1
>3 zones		70	16.9
Cavity on chest radiograph*	413		
Yes		290	70.2
No		123	29.8
Drug resistant profile	430		
Sensitive to all four drugs tested†		272	63.3
Any resistance to isoniazid		113	26.3
Any resistance to streptomycin		111	25.8
Any resistance to rifampicin		15	3.5
Any resistance to ethambutol		9	2.1
Multidrug resistance		13	3.0
MTB lineage/sublineage	413		
Modern Beijing		88	21.3
Ancient Beijing		152	36.8
Non-Beijing		173	41.9

HIV: Human immunodeficiency virus; MTB: *Mycobacterium tuberculosis*.
* The number of patients from whom available information was obtained was listed. The information on cavitary lesion was available in 413 patients, although 414 chest x-ray films were taken.
† Includes isoniazid, streptomycin, rifampicin, and ethambutol.

3.2. Sublineages of Beijing genotype strains and treatment failure

MTB genotypes were divided into three categories: modern Beijing, ancient Beijing, and non-Beijing, including East African–Indian type. Univariate analysis indicated that infection with modern Beijing MTB strains was significantly associated with treatment failure [OR = 4.15 (95% CI, 1.01–17.00)], whereas infection with ancient Beijing strains was not, when non-Beijing types were set as a reference category. Treatment failure was significantly associated with resistance to SM, RMP, INH, or MDR; positivity of smear testing after the first 2 months of treatment; and the presence of infiltrates in more than half of the lung on chest radiographs. Age, sex, presence of cavities on radiographs, HIV status, and clustering status of the MTB strains were not significantly associated with treatment failure (Table 2).

Multivariate analysis indicated that RMP resistance was significantly associated with treatment failure [aOR = 6.64 (95% CI, 1.48–29.73)]; however, SM resistance, INH resistance, and infection with the modern Beijing sublineage were not (Table 2). When we replaced RMP and INH resistance by MDR in the same model, MDR

Table 2
Results of univariate and multivariate analyses using logistic regression models on sublineages of Beijing genotype and other factors evaluated for an association with treatment failure (n = 430).

	Proportion with treatment failure (%)	Univariate		Multivariate*	
		OR	95% CI	OR	95% CI
Age (increased by one year)		1.00	0.96–1.03		
Gender					
Male	15/341 (4.4)	1.00	(Reference)		
Female	2/89 (2.3)	0.50	0.11–2.23		
MTB strain					
Other strains	3/173 (1.73)	1.00	(Reference)	1.00	(Reference)
Ancient Beijing	6/152 (3.95)	2.33	0.57–9.48	1.38	0.31–6.11
Modern Beijing	6/88 (6.82)	**4.15**	**1.01–17.00**	3.71	0.86–15.96
Resistant to SM					
No	7/319 (2.2)	1.00	(Reference)	1.00	(Reference)
Yes	10/111 (9.0)	**4.41**	**1.64–11.90**	1.77	0.46–6.76
Resistant to RMP					
No	13/415 (3.1)	1.00	(Reference)	1.00	(Reference)
Yes	4/15 (26.7)	**11.24**	**3.16–40.07**	**6.64**	**1.48–29.73**
Resistant to INH					
No	7/317 (2.2)	1.00	(Reference)	1.00	(Reference)
Yes	10/113 (8.9)	**4.30**	**1.60–11.59**	2.34	0.62–8.90
Multidrug resistance					
No	13/417 (3.1)	1.00	(Reference)		
Yes	4/13 (930.8)	**13.81**	**3.76–50.72**		
Presence of infiltrate on CXR					
≤3 zones†	10/344 (2.9)	1.00	(Reference)		
>3 zones	6/70 (8.6)	**3.13**	**1.10–8.92**		
Presence of cavity on CXR					
No	1/123 (0.8)	1.00	(Reference)		
Yes	15/290 (5.2)	6.65	0.87–50.94		
Smear at month 2‡					
Negative	9/373 (2.4)	1.00	(Reference)		
Positive	8/56 (14.3)	**6.74**	**2.48–18.30**		
HIV infection					
No	16/406 (3.9)	1.00	(Reference)		
Yes	1/22 (4.6)	1.16	0.15–9.17		
Clustered MTB strains					
No	10/271 (3.7)	1.00	(Reference)		
Yes	5/142 (3.5)	0.95	0.32–2.84		

MTB: *Mycobacterium tuberculosis*; OR: odds ratio; 95% CI: 95% confidence interval; SM: streptomycin; RMP: rifampicin; INH: isoniazid; HIV: human immunodeficiency virus; CXR: chest X-ray.
Boldfaced values indicate odds ratios and 95% CI with statistical significance ($P < 0.05$).
* In the multivariate analysis, resistance to SM, RMP, and INH, MTB strains (ancient Beijing, modern Beijing and others) were included in the logistic models.
† Zones of the lung field.
‡ Two months after starting treatment.

was also significantly associated with treatment failure [aOR = 10.23 (95% CI, 2.27–46.15)] (table not shown). Smear test results at month 2 and chest X-ray (CXR) findings may represent disease severity [30] as a result of host–pathogen interaction or phenotypic consequences of variations in MTB lineages/sublineages [31]. Therefore, we did not include them in the final model for the purposes of the present study.

3.3. Sublineages of Beijing genotype strains and TB recurrence

First, patients infected with a Beijing strain had a significantly shorter time to TB recurrence, confirmed by the log-rank test ($P = 0.0211$) (Figure 2A). Second, patients with the modern Beijing strains had a significantly shorter time to TB recurrence than patients with non-Beijing strains ($P = 0.0213$) (Figure 2B). However, no other pairwise comparisons of sublineages were statistically significant (data not shown). The numbers of patients showing recurrence during the observation period were 9 (11.3%) among the 80 patients with the modern Beijing MTB strain, 13 (9.1%) among the 143 patients with the ancient Beijing strain, and 6 (3.6%) among the 165 patients with other (non-Beijing) strains. The proportions of patients with TB recurrence within one year (12 months) after completing treatment for the prior TB episode were 11.2% (95% CI, 6.0–20.3) in those infected with the modern Beijing MTB strain, 8.5% (4.9–14.4) in those with the ancient Beijing strain, and 3.7% (1.7–8.0) with other strains.

Assuming that coexisting drug resistance might influence the effect of Beijing MTB lineages/sublineages on recurrence, we next investigated the effects of recurrence-associated factors using Cox proportional hazard models for univariate and multivariate analyses. Using the non-Beijing strains as the reference group, infection with the modern Beijing strains was significantly associated with time to TB recurrence in the univariate analysis [HR = 3.16 (95% CI, 1.13–8.89)] and the multivariate analysis with adjustment for drug resistance [aHR = 3.29 (95% CI, 1.17–9.27)]. Infection with the ancient Beijing strains was not significantly associated with time to TB recurrence (Table 3).

Resistance to RMP or INH was not significantly associated with time to TB recurrence. Resistance to SM was significantly associated with time to TB recurrence in the univariate model [HR = 2.50 (95% CI, 1.21–5.15)], but not in the multivariate model (Table 3). When we replaced RMP and INH resistance by MDR in the same model, MDR was also not significantly associated with recurrence [aHR = 3.25 (95% CI, 0.71–14.94)] (table not shown). Other characteristics, including HIV coinfection, severity on CXR, and category of patients' residential areas, were not associated with time to TB recurrence (Table 3).

4. Discussion

Our study demonstrated that RMP resistance was associated with treatment failure among patients newly diagnosed with smear-positive and culture-positive pulmonary TB. In addition, we found that infection with a modern Beijing strain was a risk factor for TB recurrence within 16 months of follow-up after completing treatment for a previous TB episode. In our study population, the prevalence of INH and SM resistance was higher among patients infected with ancient Beijing strains than among those infected with modern Beijing strains; however, INH resistance and SM resistance or infection with ancient Beijing strains were not associated with the increased risk of treatment failure or TB recurrence.

Four percent of TB patients had a treatment failure in our study, similar to 4.3% of TB patients who failed treatment in a different study conducted in southern Vietnam [32], and 5% in a multicenter trial conducted in eight centers in Africa and Asia [33]. These treatment failure rates are higher than those (2%) reported globally [1], in part because of the relatively less effective treatment regimen, 2S(E)HRZ/6HE, that was used during the study period at these study sites. The proportion of TB recurrence was 7.4% in the present study, versus 8.6% in another study conducted in northern Vietnam [34], 6.5% in southern Vietnam [32], and 5% in the multicenter trial [33] cited above. The longer follow-up period (32 months as maximum) and less strict criteria used for diagnosis of TB recurrence in the northern Vietnamese study [34] may explain the higher proportion of recurrence in that study compared with our study. Conversely, active monitoring using periodical sputum testing during the follow-up period may have improved our detection of TB recurrence, compared with the passive case finding of TB recurrence used in the study in southern Vietnam [32].

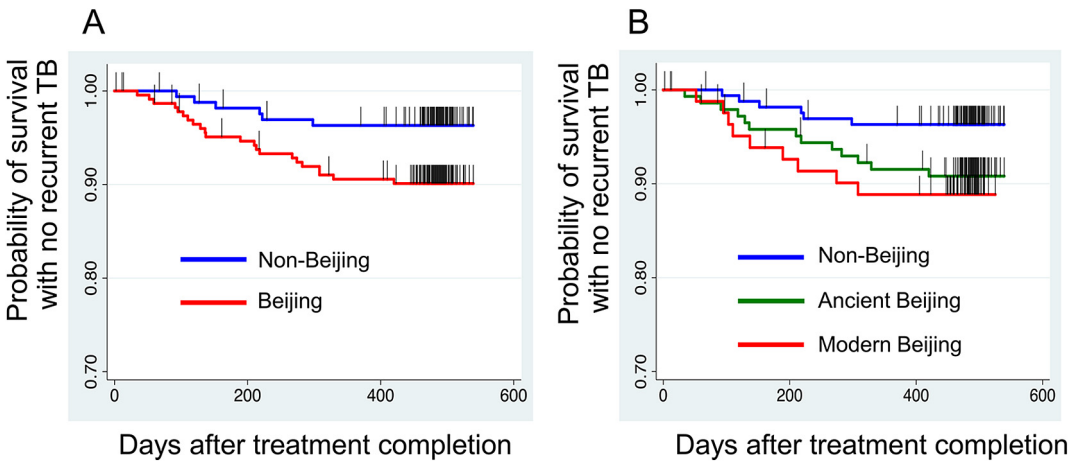

Figure 2. (A) Kaplan–Meier curves illustrating Beijing and non-Beijing MTB lineages and time to recurrence as indicated by the probability of survival with no recurrent tuberculosis, (B) Kaplan–Meier curves illustrating modern Beijing, ancient Beijing sublineages, non-Beijing lineage, and time to recurrence as indicated by the probability of survival with no recurrent tuberculosis. Time to recurrence between the Beijing and non-Beijing groups ($P = 0.0211$ by log rank test). Time to recurrence between the modern Beijing and non-Beijing groups ($P = 0.0213$ by log rank test) but not significant in other combinations, including modern Beijing and ancient Beijing sublineages (data not shown). TB: tuberculosis.

Table 3
Results of univariate and multivariate analyses using proportional hazard models on sublineages of Beijing genotype and other factors evaluated for an association with TB recurrence (n = 403).

	Proportion with recurrence (%)	Univariate		Multivariate*	
		HR	95% CI	HR	95% CI
Age (increased by one year)		1.00	0.98–1.03		
Gender					
Male	23/316 (7.3)	1.00	(Reference)		
Female	7/87 (8.1)	1.15	0.50–2.69		
MTB strain					
Other strains	6/165 (3.64)	1.00	(Reference)	1.00	(Reference)
Ancient Beijing	13/143 (9.09)	2.55	0.97–6.70	2.08	0.77–5.62
Modern Beijing	9/80 (11.25)	**3.16**	**1.13–8.89**	**3.29**	**1.17–9.27**
Resistant to SM					
No	17/306 (5.6)	1.00	(Reference)	1.00	(Reference)
Yes	13/97 (13.4)	**2.50**	**1.21–5.15**	2.33	0.97–5.61
Resistant to RMP					
No	28/394 (7.1)	1.00	(Reference)	1.00	(Reference)
Yes	2/9 (22.2)	3.63	0.86–15.26	3.1	0.66–14.55
Resistant to INH					
No	20/304 (6.6)	1.00	(Reference)	1.00	(Reference)
Yes	10/99 (10.1)	1.58	0.74–3.38	0.85	0.33–2.19
Smear at month 2†					
Negative	24/355 (6.8)	1.00	(Reference)		
Positive	6/48 (12.5)	1.84	0.75–4.50		
Clustered					
No	15/255 (5.9)	1.00	(Reference)		
Yes	13/133 (9.8)	1.64	0.78–3.45		
Presence of infiltrate on CXR					
≤3 zones‡	26/327 (8.0)	1.00	(Reference)		
>3 zones	4/62 (6.5)	0.79	0.27–2.25		
Presence of cavity on CXR					
No	11/119 (9.2)	1.00	(Reference)		
Yes	19/269 (7.1)	0.75	0.36–1.59		
HIV infection					
No	29/380 (7.6)	1.00	(Reference)		
Yes	1/21 (4.8)	0.66	0.09–4.85		

TB: tuberculosis; MTB: *Mycobacterium tuberculosis*; HR: hazard ratio; 95% CI: 95% confidence interval; SM: streptomycin; RMP: rifampicin; INH: isoniazid; HIV: human immunodeficiency virus; CXR: chest X-ray.
Boldfaced values indicate hazard ratios and 95% CI with statistical significance ($P < 0.05$).
* In the multivariate analysis, resistance to SM, RMP, and INH, MTB strains (ancient Beijing, modern Beijing and others) were included in the Cox models.
† Two months after starting treatment.
‡ Zones of the lung field.

In the present study, RMP resistance was strongly associated with treatment failure but not with recurrence. The majority of RMP-resistant strains had MDR (22/24 or 91.7%), which is a well-known risk factor for treatment failure [35]. In some resource-limited settings, drug susceptibility testing is not performed routinely for new patients before starting treatment. In those settings, application of necessary countermeasures against MDR, including a timely change of the treatment regimen, should be considered once treatment failure is suspected.

Also in the present study, active disease caused by the Beijing genotype was associated with an increased risk of TB recurrence, which was consistent with previous reports from different areas of the world [10–13]. Interestingly, modern Beijing strains exhibited a potential risk for recurrence when non-Beijing MTB genotypes were set as the reference group. Other investigators [36] have previously reported the high prevalence of modern Beijing strains among patients with a history of anti-TB treatment, suggesting that this sublineage may be associated with TB recurrence. Our study results suggest that the modern Beijing strains influenced TB recurrence, whereas the ancient Beijing strains did not. There was no significant difference in the probability of recurrent TB in patients with ancient versus modern Beijing sublineages. This result could be due to the low number of recurrent TB patients with the ancient sublineage in our study, as well as insufficient statistical power.

Virulent phenotypes of the modern Beijing sublineage strains could be differentiated by frequent drug resistance mutations and propagation, or disease development with a short latency period based on the host–pathogen interaction. In our study, the modern Beijing sublineage was actually an independent risk factor regardless of drug resistance status in the multivariate analysis. Disease caused by modern Beijing MTB strains conferred a risk of TB recurrence, despite having less frequent drug resistance than that caused by the ancient Beijing genotype. Previous reports indicate that modern Beijing strains produce lower levels of proinflammatory cytokines [37,38] and have a higher survival in macrophages [38] than ancient Beijing strains. These factors may facilitate persistent infection or reactivation from latent infection, although investigation of these properties at sublineage levels has only just begun. Nevertheless, these findings could help explain

their potential to dysregulate host defense mechanisms, leading to increased virulence and successful spread of the modern sublineage [14,15,17].

SM resistance unexpectedly exhibited a significant association with recurrence in the univariate analysis but not in the multivariate analysis. In another study conducted in southern Vietnam [13], resistance to INH was a risk factor for recurrence. Although direct evidence is lacking, this inconsistency may be due to area-dependent differences in the distribution patterns of major Beijing sublineages and their subgroups. Resistance to SM cannot be overlooked because this antibiotic is still used as a component of treatment regimens, such as 2SHRZE/1HRZE/5HRE, recommended by WHO for severe TB or retreated TB cases [39], in which relapse is often observed.

Other factors (e.g., poor treatment adherence, increased disease severity by expansion of lesions in the lung, HIV coinfection [4], diabetic status [40], and clustering status possibly indicating recent transmission) were not associated with TB recurrence by univariate analysis in our study ($P > 0.2$, data not shown). For this reason, they were not included in the multivariate analysis. Positive smear results at month 2 were significantly associated with treatment failure. This was consistent with the classical idea that persistently positive smear testing after the intensive treatment phase is a possible surrogate for treatment outcome [41].

The present study has some limitations. First, we were not able to differentiate relapse from reinfection among recurrent cases. However, we presume they were true relapse cases because most of the second TB episodes appeared in a relatively short time after cure; the median of time to recurrence was 137 days (95% CI, 110–218 days) after the end of treatment. Second, we were unable to study the host immune response to clarify the mechanism(s) underlying the association between modern Beijing sublineage and recurrence. Nevertheless, this is the first report from Vietnam on the association between MTB sublineages and unfavorable treatment outcomes, including recurrence. Future subclassification of Beijing sublineages through the analysis of genome-wide variations may help clarify the genotype–phenotype relationship and may provide an explanation for the inconsistent results previously reported. In addition, the strength of our cohort study was supported by a high proportion of patients completing treatment, followed up actively and intensively, and with clinicoepidemiological data linked to MTB lineages/sublineages with drug-resistance profiles.

In conclusion, among patients newly diagnosed with smear-positive pulmonary TB, infection with the modern Beijing sublineage was associated with recurrence, whereas infection with the ancient Beijing sublineage was not. Considering the high prevalence of circulating Beijing genotype strains and pretreatment drug resistance in the study area, multiple factors should be considered to achieve better TB management.

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