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Recurrent tuberculosis in an urban area in China: Relapse or exogenous reinfection?

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ABSTRACT

Recurrent tuberculosis is an important indicator of the effectiveness of tuberculosis control and can occur by relapse or exogenous reinfection. We conducted a retrospective cohort study on all bacteriologically confirmed tuberculosis cases that were successfully treated between 2000 and 2012 in Shanghai, an urban area with a high number but a low prevalence rate of tuberculosis cases and a low prevalence of HIV infection. Genotyping the *Mycobacterium tuberculosis* from clinical isolates was used to distinguish between relapse and reinfection. In total, 5.3% (710/13,417) of successfully treated cases had a recurrence, a rate of 7.55 (95% CI 7.01–8.13) episodes per 1000 person-years, more than 18 times the rate of tuberculosis in the general population. Patients who were male, age 30–59, retreatment cases, had cavitation, diabetes, drug-resistant or multidrug-resistant tuberculosis in their initial episode of tuberculosis, were at high risk for a recurrence. Among 141 recurrent cases that had paired isolates, 59 (41.8%) had different genotypes, indicating reinfection with a different strain. Patients who completed treatment were still at high risk of another episode of tuberculosis and exogenous reinfection contributed a significant proportion of the recurrent tuberculosis cases. Targeted control strategies are needed to prevent new tuberculosis infections in this setting.

1. Introduction

Tuberculosis still poses a huge threat to global health, and now ranks alongside human immunodeficiency virus (HIV) infection as a leading cause of death worldwide with an estimated 1.5 million tuberculosis deaths in 2014 [1]. Although tuberculosis is curable under a standard treatment of combination regimens, some patients who complete an adequate course of treatment still develop a subsequent episode, or recurrent tuberculosis. Patients with recurrent tuberculosis often require an extra round of treatment with a regimen that takes a longer time to complete, leading to a lower rate of treatment success and further contributing to additional transmission of strains of *Mycobacterium tuberculosis* (*M. tuberculosis*) [2,3].

Recurrent tuberculosis can occur by relapse of the original infecting strain of *M. tuberculosis*, or exogenous reinfection with a

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new strain [4,5]. Comparisons of genotype patterns between paired isolates from different episodes of tuberculosis can differentiate between relapse and exogenous reinfection, a distinction that has important implications for tuberculosis control, assessment of anti-tuberculosis treatment, evaluation of effectiveness of case management and endpoints in clinical trials [6,7]. The proportion of reported recurrence caused by exogenous reinfection varied widely in different studies [8]. Such variations may be explained by differences in the incidence of tuberculosis infection and the presence of risk factors that increase the likelihood that an infection will occur and progress to active disease. In particular, HIV infection was widely reported to be risk factor for both recurrence and exogenous reinfection in many settings [2,6,7]. In areas with a high prevalence of HIV infection, for example in South Africa and Malawi, tuberculosis recurrence due to exogenous reinfection was common in HIV infected populations but rare in HIV non-infected populations [9,10]. One study from a setting in South India also reported a high rate (up to 88%) of reinfection among recurrent cases in a group with a prevalence of HIV infection [11]. However, recurrence and its cause in settings with a low prevalence of HIV infection has not been well described.

Despite the substantial progress in tuberculosis control, China is still one of the most high-burden countries globally [1,12]. Although, most patients are cured with a low treatment failure rate [12], there is limited data on recurrence following completion of treatment. A better understanding of the rate of recurrent tuberculosis and its associated risk factors is crucial to target interventions to reduce the frequency of this disease. We conducted a retrospective cohort study in Shanghai, an urban setting in China with a low prevalence of HIV infection [13–15], to estimate the rate of recurrence, to determine the risk factors associated with recurrence, and to estimate the frequency of exogenous reinfection with a different *M. tuberculosis* strain.

2. Patients and methods

2.1. Study setting and population

Shanghai is the most populous city in China and had an estimated 24.3 million inhabitants in 2015. We conducted a retrospective cohort study in the local resident population using all tuberculosis cases that were bacteriologically confirmed (by smear or culture) and reported to the Shanghai Municipal Center for Disease Control and Prevention (Shanghai CDC) from January 1, 2000 to December 31, 2012. The estimated tuberculosis incidence rate among local residents in Shanghai was 40 per 100,000 population in 2000 and dropped to 26.5 per 100,000 in 2015, a relatively low incidence rate compared to other areas in China [16]. The prevalence of HIV-infection in Shanghai is low among the general population (approximately 0.6 cases/100,000 persons per year) and tuberculosis patients (<0.1%) [13,14].

In the tuberculosis program in Shanghai, individuals with tuberculosis symptoms from general hospitals and community health centers are referred to designated tuberculosis hospitals/clinics for confirmation and diagnosis by sputum smear test and bacterial culture. Since the 1990s, Shanghai established a surveillance system and mandatory routine reporting of tuberculosis cases. The routine surveillance system records demographic, clinical and laboratory information on every individual diagnosed with tuberculosis. Treatment regimens of new and previously treated tuberculosis cases were according to the National Tuberculosis Control Program (NTP) [17]. Both the intensive phase and the continuation phase of anti-tuberculosis therapy can be extended, depending on the radiological and bacteriological data that are available and the clinical judgment of the treating physician.

2.2. Data linkage and definitions

Data linkage using the routine surveillance system identified recurrent cases among all tuberculosis cases reported between 2000 and 2012. Cases were automatically compared and ranked by the degree of similarity of personal identifiers, including patient's identification number, names, gender and home or work address. Cases with same patient identification that were mismatched in only one to two characters of the name were manually checked to determine whether there was mistyping (usually for Chinese characters with similar pronunciations or shapes). Cases with the same name but different patient identification numbers were also manually checked to rule out any misclassification.

The present study included all local residents, but not migrants, aged ≥15 years old with bacterially confirmed (either sputum smear-positive or culture-positive) pulmonary tuberculosis who were successfully treated in their initial episode of tuberculosis. Tuberculosis recurrence was defined according to the China National Tuberculosis Program (NTP) management guidelines as any new bacterially confirmed tuberculosis diagnosis in a patient who had a prior episode of tuberculosis with treatment success [17]. Treatment success includes two categories: "cured" was defined as a negative sputum smear at the end of treatment and on one previous occasion among patients initially sputum smear-positive; and "treatment completed" for patients who completed treatment without bacteriological proof of cure by sputum smear and without evidence of treatment failure.

Because of the retrospective study design, there was no active follow-up for all of the cases. In order to calculate the rate of recurrent tuberculosis among all reported cases, the passive follow-up time was defined as the time elapsed since the end of the initial tuberculosis treatment until an event (recurrence) or the censored date (December 31, 2013). Retreatment cases were defined as patients who had a prior history of ≥30 days of anti-tuberculosis treatment before the first episode. Diabetes status was based on the diagnostic information in the medical records. Multidrug-resistant tuberculosis (MDR-TB) was defined as resistance to at least isoniazid and rifampin.

2.3. Classification of relapse and reinfection

Paired isolates from patients with recurrent tuberculosis were sub-cultured from the frozen stock using Löwenstein-Jensen (L-J) solid slants. Genomic DNA was obtained from isolates by the boiled lysis method [18]. Genotyping was performed using the 9 + 3 variable number of tandem repeats (VNTR) loci set as previous described [19]. Cross-contamination was identified and excluded if two or more isolates from different patients were processed on the same day in the laboratory and shared the same genotype [18]. A relapse was considered to occur when the molecular genotypes for the strains from the sequential episodes matched exactly. Exogenous reinfection was defined if the genotype patterns from a patient's sequential isolates were different.

2.4. Statistical analysis

We used the host and mycobacterial characteristics, e.g. age, drug susceptibility or resistance, reported during a patient's first episode of tuberculosis to tests for associations with recurrent tuberculosis. We estimated the hazards of recurrent tuberculosis using the Kaplan-Meier method. Subgroups of interest were compared using the log rank test. Cox proportional hazard models were used to investigate the risk factors associated with recurrent tuberculosis. Only the first episode of recurrence was included in the risk factor analysis. Unadjusted hazard ratios (HR) for

demographic, microbiological and clinical factors were calculated from univariable analysis. Adjusted hazard ratios were estimated by multivariable analysis. The final model was adjusted for sex, age and those variables with p values less than 0.2 from the univariable analysis. Results with a p value less than 0.05 were considered significant in the final multivariable model. The Schoenfield residuals test was used to test the proportional hazard assumption in the Cox model. Survival analysis was used to compare the distributions of relapse and exogenous reinfection tuberculosis cases over time since treatment completion of the index episode. We used the Kolmogorov-Smirnov test to test for a difference in the observed distributions of different group of recurrence (i.e., relapse and exogenous reinfection). All analyses were performed using Stata version SE 13 (Stata Corp., College Station, Texas, USA).

2.5. Ethics statement

The study protocol was approved by the Institutional Reviewed Board of the Institutes of Biomedical Sciences, Fudan University (Ethics approval No. 74) and the Shanghai CDC. The study was exempted from full institutional review by the Institutional Review Board of the University of California, Davis, because their work involved the study of existing data, documents and records for participants that could not be identified.

3. Results

3.1. Frequency and description of recurrences

During the study period of January 1, 2000 through December 31, 2012, there were 15,812 bacteriologically confirmed active pulmonary tuberculosis cases among the resident population in Shanghai, and 84.9% (13,417/15,812) of them were successfully treated in this period. Among them, 5.3% (710/13,417, 95% CI, 4.92–5.68) had a bacteriologically confirmed case of at least one another subsequent episode, i.e. recurrent tuberculosis (Fig. 1). Among the 710 recurrent cases, 698 had one recurrent episode and 12 cases had two or more recurrent episodes during the study period. Overall, the rate of recurrent tuberculosis during a total of 93939.3 person years (PYs) was 7.55 (95% CI, 7.01–8.13) recurrences per 1000 PYs. The median time from the completion of first episode to recurrent tuberculosis was 1.3 years (IQR 0.6–2.8 years).

3.2. Risk factors associated with recurrence

We used multivariate Cox analysis to identify the risk factors recorded at the initial treatment episode that were associated with the 710 recurrent tuberculosis episodes (Table 1). The highest rate of recurrence was found among cases with MDR-TB (23.4 recurrences per 1000 PYs). The risk factors that were independently associated with a greater risk of recurrence relative to each baseline group were male gender (adjusted HR [aHR] 1.40, 95% CI 1.11–1.77), age 30–59 years (aHR 1.24, 95% CI 1.03–1.47), cavitation on the initial chest radiograph (aHR 1.27, 95% CI 1.06–1.51), diabetes (aHR 1.42, 95% CI 1.13–1.79), being a retreatment cases (aHR 1.82, 95% CI 1.49–2.23), being sputum smear positive (aHR 1.59, 95% CI 1.19–2.13), and having non-MDR drug resistance (aHR 1.52, 95% CI 1.23–1.88) and MDR-TB (aHR 2.90, 95% CI 2.20–3.84).

3.3. Classification of relapse and exogenous reinfection

Among the 710 bacteriologically confirmed recurrent tuberculosis cases, 514 were culture positive in both episodes (Fig. 1). One-hundred and forty-one of these 514 patients (27.4%, 141/514) had paired isolates that were successfully re-cultured for genotyping. Among the remaining 357 patients without paired isolates available, 305 (85.4%) were from 2000 to 2007, a period that lacked consistent storage conditions that explained why many isolates could not be re-cultured. However, there was no significant difference between culture-positive recurrent cases with and without paired isolates, except patients ≥60 years old were less likely to have paired isolates available (Supplementary Table 1). Among the 141 patients with genotyped paired isolates, 58.2% (82/141) had identical genotypic patterns, indicating a relapse occurred, and 41.8% (59/141) had different genotypic patterns between the isolates of initial and subsequent episodes (Fig. 1), indicating exogenous reinfection with a new strain of *M. tuberculosis*. Among the recurrent cases with exogenous reinfection, the distribution of the VNTR loci difference between strains from the first and second episodes ranged from one locus to more than ten loci (Supplementary Table 2).

Additionally, we identified one recurrent case that had at least two different strains of *M. tuberculosis* present in the isolate from the initial episode of tuberculosis. The single strain found at the recurrent episode was identical to the minority strain present at the initial episode in this patient, suggesting a mixed infection with multiple strains of *M. tuberculosis* in the initial episode and one of the sub-populations of *M. tuberculosis* persisted after the initial

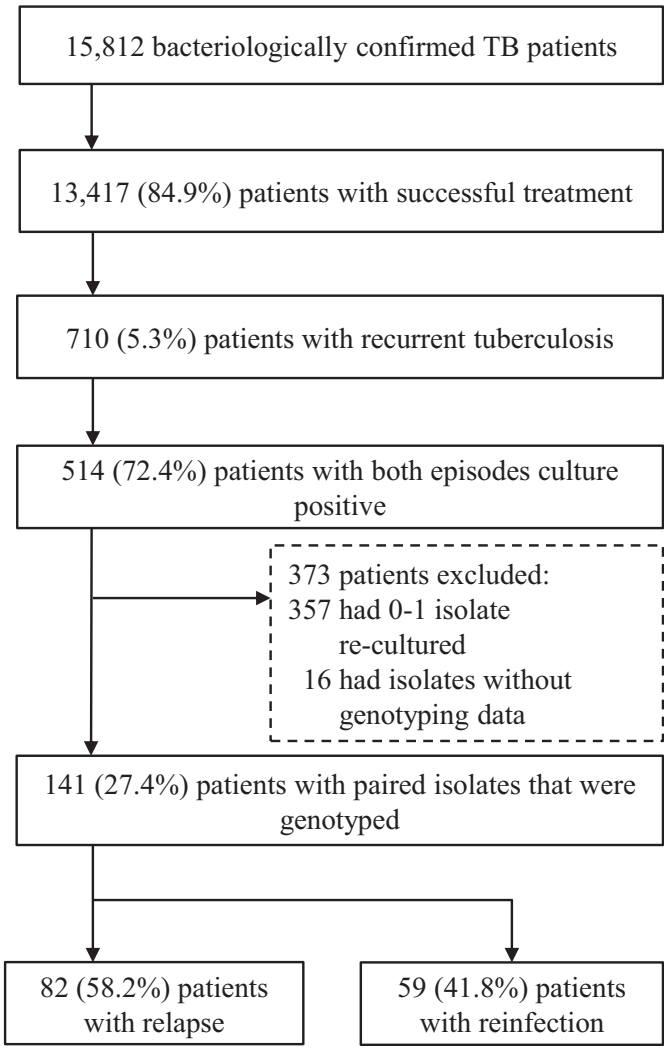

Fig. 1. Flow chart of tuberculosis cases included and excluded from the study. TB, tuberculosis.

Table 1
Incidence rate and hazard ratios by characteristics of recurrent tuberculosis cases, by univariate and multivariable analysis, Shanghai, 2000–2012.

Characteristic	Category	Number of reported cases	Number of Recurrent events	Incidence per 1000 years	Univariate		Multivariable[a]	
					HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
All		13,417	710	7.55 (7.01–8.13)				
Sex	Female	3148	122	5.49 (4.57–6.56)	1.00		1.00	
	Male	10,269	588	8.20 (7.55–8.88)	1.49 (1.22–1.81)	<0.001	1.40 (1.10–1.75)	<0.01
Age groups, years	15–29	1813	57	4.65 (3.52–6.02)	1.00		1.00	
	30–59	5945	365	8.76 (7.89–9.70)	1.93 (1.46–2.55)	<0.001	1.38 (1.01–1.88)	0.04
	≥60	5659	288	7.20 (6.39–8.08)	1.60 (1.20–2.13)	0.001	1.14 (0.83–1.58)	
Cavity on chest radiograph	No	8473	394	6.35 (5.74–7.01)	1.00		1.00	
	Yes	4594	288	9.98 (8.87–11.19)	1.43 (1.23–1.67)	<0.001	1.27 (1.06–1.51)	<0.01
	Unknown	350	28	9.21 (6.13–13.28)	1.60 (1.09–2.35)	0.01	1.70 (1.08–2.67)	0.02
Diabetes	No	11,381	586	7.15 (6.58–7.75)	1.00		1.00	
	Yes	1515	111	9.97 (8.69–12.70)	1.43 (1.17–1.75)	0.001	1.40 (1.13–1.76)	<0.01
	Unknown	521	13	8.89 (4.73–15.14)	0.65 (0.37–1.12)	0.121	0.65 (0.34–1.26)	0.20
Status at first episode of tuberculosis	New case	11,512	541	6.78 (6.22–7.38)	1.00		1.00	
	Retreated case	1905	169	11.94 (10.21–13.86)	1.84 (1.55–2.19)	<0.001	1.80 (1.45–2.20)	<0.001
Sputum smear status	Negative	2029	70	4.96 (3.87–6.26)	1.00		1.00	
	Positive	11,334	637	8.04 (7.42–8.68)	1.61 (1.26–2.07)	<0.001	1.59 (1.19–2.14)	<0.01
	Unknown	54	3	5.31 (1.09–12.47)	1.37 (0.43–4.37)	0.588	1.63 (0.22–11.83)	0.62
DST profile (n = 9365)	Pan-susceptible	7364	367	7.02 (6.31–7.77)	1.00		1.00	
	Non-MDR DR	1597	118	12.09 (10.02–14.46)	1.58 (1.29–1.96)	<0.001	1.52 (1.23–1.88)	<0.001
	MDR	404	59	23.4 (17.88–30.12)	3.12 (2.27–4.11)	<0.001	2.90 (2.20–3.84)	<0.001

[a] The multivariable model adjusted for sex, age and all the other variables in the table. Abbreviations: HR, hazards ratio; DST, drug susceptible testing; MDR, multidrug resistance; non-MDR DR, drug resistance to any of the four first line drugs (isoniazid, rifampin, ethambutol and streptomycin), but not MDR.

completion of treatment.

We further compared the characteristics and estimated the time to relapse versus exogenous reinfection. Fig. 2 shows the time since the completion of treatment for patients with a known relapse or reinfection. There was a significantly shorter time to recurrent tuberculosis caused by a relapse compared to exogenous reinfection (Kolmogorov-Smirnov test for difference in the observed distributions, $P = 0.02$; Fig. 2). The proportion of recurrent cases due to exogenous reinfection increased as the time since completion of therapy increased. In addition, patients with diabetes at the initial episode had a significantly shorter time to recurrence caused by exogenous reinfection, compared to a recurrence caused by relapse (Supplementary Figure).

3.4. Risk factors associated with relapse and exogenous reinfection

We first compared the characteristics between recurrence patients with relapse or exogenous reinfection and non-recurrence patients (Supplementary Table 3). Compared to non-recurrence patients, patients of male gender, age 30–59 years old, retreatment, sputum smear-positive, cavity on chest radiograph, and MDR-TB at the first episode were at higher risk for relapse. Retreatment and MDR-TB were also significantly associated with exogenous reinfection, compared to those non-recurrence patients.

We then assessed the rate and multivariate analysis of hazard ratios of characteristics of relapse and exogenous reinfection cases (Tables 2 and 3). In the multivariable analysis, male patients were still at high risk for a relapse (adjusted hazards ratio, aHR 3.14, 95% CI 1.43–6.84, P = 0.004) but not reinfection; patients with MDR-TB were at increased risk relapse (aHR 3.15, 95% CI 1.59–6.26, $P < 0.01$) but just slightly at risk of exogenous reinfection (aHR 2.34, 95% CI 0.98–5.58, $P = 0.054$); and patients with a retreatment history were at risk of relapse and reinfection (Tables 2 and 3).

4. Discussion

Of 13,417 bacteriologically confirmed active pulmonary tuberculosis cases after treatment completion in Shanghai during 2000–2012, at least 5.3% (95% CI 4.92–5.68) of the notified tuberculosis patients experienced one or more recurrences of tuberculosis. The overall rate of recurrent tuberculosis in this population was 7.55 cases per 1000 person years, or 755 cases per 100,000 population. The hazard of recurrent tuberculosis was significantly higher for tuberculosis patients who were male, age 30–59, retreatment cases, had cavitation, diabetes, and drug-resistant or MDR-TB in their initial episode of tuberculosis. Notably, even in this setting with a low prevalence of HIV infection, 42% of the recurrent tuberculosis was attributed to exogenous reinfection with a different *M. tuberculosis* strain.

In a systematic review, the median recurrence rates varied from 1780 (1000–4000) to 7850 (980–11900) episodes per 100,000 PYs of follow-up for low and high tuberculosis incidence countries, respectively [20]. In a study from England and Wales from 1998 to 2006, the reported recurrence rate among culture-positive tuberculosis patients was 660 cases per 100,000 PYs [21]. In Barcelona, Spain, the rate of recurrent tuberculosis in all patients was 341

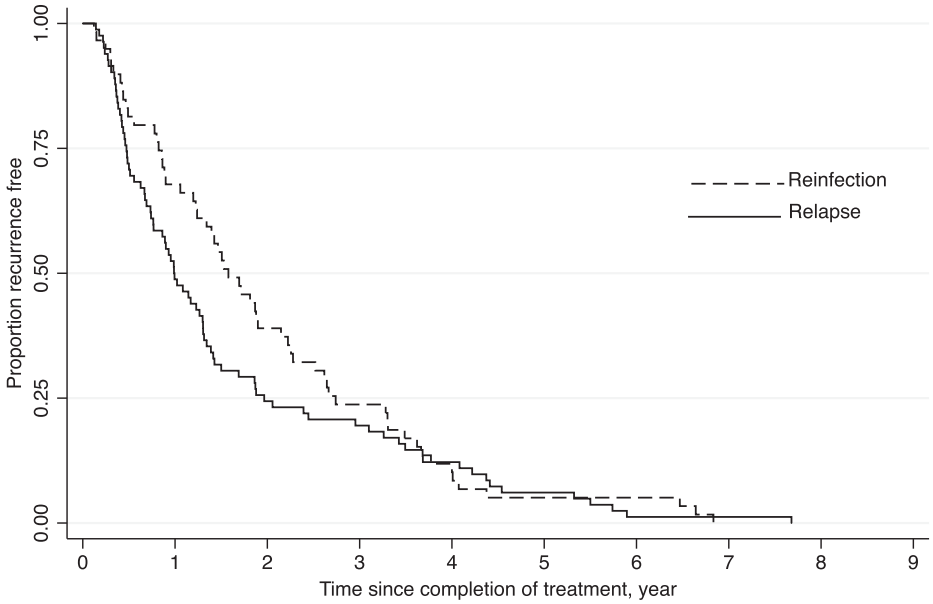

Fig. 2. Kaplan-Meier survival estimates for recurrent tuberculosis due to relapse (n = 82, solid line) and exogenous reinfection (n = 59, dashed line). Only the patient with a known relapse or reinfection were included. (Kolmogorov-Smirnov test for difference in relapse versus exogenous reinfection, $P = 0.02$).

cases per 100,000 PYs, 13 times higher than the incident tuberculosis rate in the general population [22]. Although the incidence rate of bacteriologically confirmed recurrent tuberculosis in the present study (755 cases per 100,000 person years) was within the rate range reported in the low incidence regions [21–23], differences in enrollment criteria, patient follow-up time, the data collected on putative risk factors for recurrent tuberculosis, and the length of the study varied between studies and limit the

Table 2
Incidence rate and hazard ratios by characteristics of relapse tuberculosis cases, by univariate and multivariable analysis, Shanghai, 2000–2012.

Characteristic	Category	Number of reported tuberculosis cases	Number of relapse events	Incidence per 10,000 years	Univariate		Multivariable[a]	
					HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
All		12,848	82	8.84 (7.04–10.98)				
Sex	Female	3046	7	3.18 (1.28–6.56)	1.00		1.00	
	Male	9802	75	10.60 (8.34–13.29)	3.33 (1.54–7.23)	<0.01	3.14 (1.44–6.85)	0.004
Age groups, years	≤29	1775	9	7.39 (3.38–14.03)	1.00		1.00	
	30–59	5661	52	12.67 (9.46–16.61)	1.78 (0.88–3.62)	0.10	1.32 (0.64–2.71)	0.44
	≥60	5412	21	5.32 (3.29–8.13)	0.75 (0.34–1.64)	0.48	0.54 (0.24–1.21)	0.13
Cavity on chest radiograph	No	8154	40	6.52 (4.66–8.88)	1.00			
	Yes	4367	38	13.37 (9.46–18.34)	1.84 (1.18–2.87)	<0.01		
	Unknown	327	4	13.42 (3.66–34.34)	2.34 (0.87–6.54)	0.10		
Diabetes	No	10,912	67	8.28 (6.42–10.52)	1.00			
	Yes	1426	14	13.51 (7.39–22.65)	1.58 (0.89–2.80)	0.12		
	Unknown	510	1	6.89 (0.14–38.31)	0.40 (0.06–2.89)	0.36		
Status at first episode of tuberculosis	New case	11,074	61	7.74 (5.92–9.94)	1.00		1.00	
	Retreated case	1774	21	15.11 (9.36–23.10)	2.08 (1.27–3.43)	<0.01	2.30 (1.36–3.85)	0.002
Drug resistance profile (n = 8960)	Pan-susceptible	7093	54	10.45 (7.84–13.63)	1.00		1.00	
	Non-MDR DR	1506	18	18.70 (11.09–29.54)	1.63 (0.96–2.79)	0.07	1.42 (0.84–2.44)	0.19
	MDR	361	10	41.20 (19.78–75.68)	3.73 (1.90–7.32)	<0.001	3.16 (1.60–6.26)	0.001

[a] The multivariable model adjusted for sex, age, treatment history and drug resistance profiles. Abbreviations: DST, drug susceptible testing; MDR, multidrug resistance; HR, hazards ratio; Non-MDR DR, drug resistance to any of the four first line drugs (isoniazid, rifampin, ethambutol and streptomycin), but not MDR.

Table 3
Incidence rate and hazard ratios by characteristics of reinfection tuberculosis cases, by univariate and multivariable analysis, Shanghai, 2000–2012.

Characteristic	Category	Number of reported tuberculosis cases	Number of relapse events	Incidence per 10,000 years (95% CI)	Univariate		Multivariable[a]	
					HR (95% CI)	*P* value	Adjusted HR (95% CI)	*P* value
All		12,848	59	6.36 (4.84–8.21)				
Sex	Female	3046	13	5.91 (3.15–10.11)	1.00		1.00	
	Male	9802	46	6.50 (4.76–8.67)	1.10 (0.59–2.04)	0.71	1.12 (0.58–2.14)	0.72
Age groups, years	≤29	1775	10	8.21 (3.94–15.10)	1.00		1.00	
	30–59	5661	29	7.07 (4.73–10.15)	0.89 (1.46–2.55)	0.75	0.82 (0.38–1.76)	0.61
	≥60	5412	20	5.06 (3.09–7.82)	0.64 (1.20–2.13)	0.25	0.52 (0.22–1.76)	0.12
Cavity on chest radiograph	No	8154	35	5.70 (3.98–7.94)	1.00			
	Yes	4367	23	8.09 (5.13–12.14)	1.28 (0.76–2.18)	0.34		
	Unknown	327	1	3.35 –	0.65 (0.09–4.79)	0.67		
Diabetes	No	10,912	50	6.18 (4.59–8.15)	1.00			
	Yes	1426	8	7.71 (3.33–15.20)	1.20 (0.57–2.54)	0.62		
	Unknown	510	1	6.87 –	0.56 (0.07–4.13)	0.08		
Status at first episode of tuberculosis	New case	11,074	42	5.32 (3.84–7.20)	1.00		1.00	
	Retreated case	1774	17	12.23 (7.12–19.58)	2.43 (1.38–4.27)	0.002	2.96 (1.62–5.39)	0.001
Drug resistance profile (n = 8960)	Pan-susceptible	7093	42	8.13 (5.86–10.98)	1.00		1.00	
	Non-MDR DR	1506	9	9.35 (4.28–17.75)	1.06 (0.52–2.19)	0.86	0.95 (0.46–1.98)	0.91
	MDR	361	6	24.72 (9.08–53.73)	2.90 (1.23–6.82)	0.01	2.34 (0.98–5.58)	0.05

[a] The multivariable model adjusted for sex, age, treatment history and drug resistance profiles. Abbreviations: DST, drug susceptible testing; MDR, multidrug resistance; HR, hazards ratio; non-MDR DR, drug resistance to any of the four first line drugs (isoniazid, rifampin, ethambutol and streptomycin), but not MDR.

comparisons. Notably, the incidence of recurrent tuberculosis in the current study was 18.9 times higher among successfully treated tuberculosis patients than the estimated incidence of tuberculosis among the general population in Shanghai (40 per 100,000 population), higher than the rate differences (3–13 times) reported in other studies [21,22,24,25]. These findings imply that patients with completed treatment can still be at high risk for another tuberculosis episode.

The proportion of recurrent tuberculosis cases caused by exogenous reinfection also varied widely, from 0 to 100%, in previous studies [8,26–28]. A previous study in Shanghai from 1999 to 2004 with a smaller sample size reported that 62% (32/52) of recurrent tuberculosis cases were caused by exogenous reinfection [15]. Most of the patients (90%, 47/52) were also included in present study. With the larger sample size and longer study period from 2000 to 2012, exogenous reinfection still contributed approximately 41.8% (95% CI, 33.6%–50.4%) of the recurrent tuberculosis cases. Such a high proportion of exogenous reinfection among recurrent cases still reflects a high force of tuberculosis infection in the community. A population-based molecular epidemiology study in Shanghai reported at least 30% of the tuberculosis cases resulted from recent transmission of *M. tuberculosis* [18], also indicating a considerable infection force of *M. tuberculosis* in this area. Other studies reported that exogenous reinfection is more likely occurred among people with HIV infection [2,5,9,10,25,26]. However, our results from Shanghai suggest that reinfection can also occur frequently in a setting with a low prevalence of HIV infection.

Additionally, recurrent tuberculosis due to relapse was more likely to occur within three years after completion of therapy, and recurrent tuberculosis caused by exogenous reinfection occurred with increasing time since completion of therapy. The dynamic distributions of occurrence time of these two mechanisms have important implications for post-treatment control strategies and clinical trials study design [5]. Similar findings were also reported in previous studies from South Africa and the United States [5,9,29]. The findings from settings of both high and low prevalence of HIV infection indicate that exogenous reinfection caused late recurrence, regardless of the prevalence of HIV infection in the setting.

The highest reported rate of recurrent tuberculosis was among MDR-TB patients compared to drug susceptible patients (23.4 recurrent cases per 1000 person years) and could be due to the complexity and length of anti-tuberculosis treatment for MDR cases. MDR-TB also increased the risk for relapse in the present study, indicating that many of the MDR-TB cases may not be truly cured by the treatment regimens available, and the surviving mycobacteria replicate and grow to cause disease again. Because of the lengthy treatment time and infectious status of drug-resistant patients, there needs to be particular attention to the case management of drug-resistant and MDR-TB cases. The health system needs to ensure the completion of an appropriate treatment regimen for bacterially confirmed drug-resistant tuberculosis patients, with confirmation based on bacteriological cure. In general, diabetes is considered as a common risk factor associated with tuberculosis and it can also increase the risk of poor treatment outcomes, including recurrent TB, as reported in present and several previous studies [30–32]. Considering the overlapping

burdens of tuberculosis and diabetes in China [33], the co-epidemics warrants further studies.

Our study has several limitations. First, we conducted a retrospective cohort study using routinely collected information and specimens. Some cases of recurrent tuberculosis were likely missed because the patient died or moved out of the city during the study period, which could lead to an underestimate of the true rates of recurrent tuberculosis. Secondly, less than 30% of the recurrent cases had paired isolates available for genotyping analysis due to the lack of consistent storage conditions in the earlier years, possibly introducing a sampling bias. However, patients with and without paired isolates differed only by age (Supplementary Table 1). Additionally, the definition of relapse and exogenous reinfection was based on VNTR genotyping and there may be some misclassification. For example, individuals may be reinfected after continued exposure with the same genotypic strains in a transmission chain or a household. We also cannot rule out the possibility that mixed infection, rather than exogenous reinfection, occurred in the initial episode and led to recurrent tuberculosis [5,34], and we did observe one case of mixed infection in the study. Recurrent tuberculosis detected by paired isolates that differ by one or more VNTR loci could result from the mycobacteria's micro-evolution within the host, rather than exogenous reinfection. Further implementation of whole genome sequencing analysis will provide more discriminatory power to define relapse and reinfection, and to understand the underlying population diversity of *M. tuberculosis* within individual hosts [35]. Finally, we did not obtain the HIV status of the individual recurrent tuberculosis patients, although the prevalence of HIV in Shanghai ie relatively low.

In summary, the rate of recurrent tuberculosis in Shanghai after successful treatment was relatively low, but was 18 times higher than the incidence rate of tuberculosis in the general population. Despite the relatively low incidence rate of tuberculosis and the low prevalence of HIV infection in this setting, exogenous reinfection still caused a significant proportion of the recurrent tuberculosis cases. Targeted interventions that improve case management, treatment and cure rates, and reduce the transmission of *M. tuberculosis* should be considered.

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The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the paper. Dr. Qian Gao had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflict of interest statement

We declare that we have no conflict of interest.

Author contribution

X.S. and C.Y. participated in the study design, management and analysis of data, and initially drafted the paper. J.W., J.T., M.G., L.W. and Y.L. participated in the data collection, organized and carried out the laboratory work. J.W. and T.L. contributed to the re-culture and genotyping of the isolates. S.L., X.G., Z.W. and Q.P. contributed to the management of demographic, clinical and epidemiological data collection. J.M., K.D., Z.Y. and Q.G. participated in study design, data analysis, manuscript draft and revisions, and coordinated the project. All authors reviewed and approved the final version.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tube.2017.01.007.

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