Transmission of *Mycobacterium tuberculosis* in China: A Population-Based Molecular Epidemiologic Study

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(See the Editorial Commentary by Arend and Soolingen on pages 228–32.)

Background. Understanding the transmission of *Mycobacterium tuberculosis* is essential for the development of efficient tuberculosis control strategies. China has the second-largest tuberculosis burden in the world. Recent transmission and infection with *M. tuberculosis*, particularly drug-resistant strains, may account for many new tuberculosis cases.

Methods. We performed a population-based molecular epidemiologic study of pulmonary tuberculosis in China during 1 July 2009 to 30 June 2012. We defined clusters as cases with identical variable number tandem repeat genotype patterns and identified the risk factors associated with clustering, by logistic regression. Relative transmission rates were estimated by the sputum smear status and drug susceptibility status of tuberculosis patients.

Results. Among 2274 culture-positive tuberculosis patients with genotyped isolates, there were 705 (31.0%) tuberculosis patients in 287 clusters. Multidrug-resistant (MDR) tuberculosis (adjusted odds ratio [aOR], 1.86; 95% confidence interval [CI], 1.25–2.63) and infection with a Beijing family strain (aOR, 1.56; 95% CI, 1.23–2.96) were associated with clustering. Eighty-four of 280 (30.0%) clusters had a putative source case that was sputum smear negative, and 30.6% of their secondary cases were attributed to transmission by sputum smear–negative patients. The relative transmission rate for sputum smear negative compared with sputum smear–positive patients was 0.89 (95% CI, .68–1.10), and was 1.51 (95% CI, 1.00–2.24) for MDR tuberculosis vs drug-susceptible tuberculosis.

Conclusions. Recent transmission of *M. tuberculosis*, including MDR strains, contributes substantially to tuberculosis disease in China. Sputum smear–negative cases were responsible for at least 30% of the secondary cases. Interventions to reduce the transmission of *M. tuberculosis* should be implemented in China.

Keywords. tuberculosis; molecular epidemiology; recent transmission; China.

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Tuberculosis remains a major threat to global health, and is a leading cause of death in many developing countries. Infection with *Mycobacterium tuberculosis* leads to tuberculosis disease by 2 main mechanisms: infection and rapid progression to disease from a recent transmission event, and reactivation from latent tuberculosis due to a remote infection event and progression to disease [1–4]. It is important to distinguish between these 2

mechanisms of disease, because each requires different prevention and control strategies.

The development of molecular epidemiology has allowed researchers to assess and quantify the recent transmission and reactivation of *M. tuberculosis* in different settings, and to identify the clinical and social demographic factors associated with recent transmission [1–5]. Patients with mycobacterial isolates that have identical genotypes were assigned to clusters and were assumed to be caused by recent transmission, whereas those with a unique genotype represented reactivation of a remote infection [6].

China has a serious tuberculosis epidemic, with 1.4 million prevalent tuberculosis cases and 130 000 deaths among tuberculosis cases annually [7]. Currently, the tuberculosis control program focuses on the proportion of patients with infectious tuberculosis disease who are cured by the end of their treatment regimen. However, the prevalence of tuberculosis in a population is also determined by the incidence of new tuberculosis cases and the duration of infectiousness [8]. As the duration of infectiousness of undiagnosed, untreated individuals increases, so does the likelihood that *M. tuberculosis* will transmit to others. Although China has made significant achievements tackling the tuberculosis epidemic during the last few decades [9], the prevalence of all pulmonary tuberculosis did not significantly decrease (from 466/100 000 in 2000 to 459/100 000 in 2010) [10]. Meanwhile, the relative contribution of recent transmission of *M. tuberculosis* in China is unclear.

We conducted a population-based molecular epidemiologic study of pulmonary tuberculosis in 5 field sites in China to estimate the magnitude of tuberculosis cases that were attributable to recent transmission of *M. tuberculosis*, to identify the risk factors associated with recent transmission, and to estimate relative transmission rates.

METHODS

Study Population

We performed a population-based molecular epidemiologic study in 5 field sites in China from 1 July 2009 to 30 June 2012. In each of the 5 provinces, 1 county was selected as the study site (Supplementary Figure 1). The 5 sites represent geographical areas and populations with different tuberculosis burdens and socioeconomic levels based on China's census system [10]. At each site, passive case finding was used to identify patients aged ≥15 years with suspected pulmonary tuberculosis with symptoms, including cough for at least 2 weeks, fever, chest pain, weight loss, night sweats, and abnormal chest radiograph, based on the guidelines of the Chinese National Center for Disease Control and Prevention (CDC) [9]. Community or village physicians routinely identified and referred patients with suspected tuberculosis to a designated tuberculosis hospital or to the county CDC for diagnosis. Individuals who provided written informed consent were enrolled. The study protocol was approved by the institutional review board of the Institutes of Biomedical Sciences (protocol review No. 43), Fudan University.

Laboratory Procedures

For suspected tuberculosis, 3 sputum samples collected at different time points (spot, early morning, and night) were examined for acid-fast bacilli (AFB), and 2 of them were used for Lowenstein-Jensen culture. Sputum induction was used for patients who had trouble producing a sputum sample spontaneously.

All of the *M. tuberculosis* isolates were sent to the provincial CDC for drug susceptibility testing to detect resistance to rifampin (RIF) and isoniazid (INH) using the proportion method on Lowenstein-Jensen medium at the following concentrations: RIF 40 μg/mL and INH 0.2 μg/mL [11]. Multidrug-resistant (MDR) tuberculosis was defined as resistance to at least INH and RIF.

Bacterial genomic DNA was obtained from isolates by the boiled lysis method [12]. The Beijing genotype is the most prevalent family of *M. tuberculosis* strains in China [13]. We used a set of variable number tandem repeats (VNTR), which was optimized for both Beijing strains and other strains in China, had high discriminatory power comparable to the IS6110–restriction fragment length polymorphism method [14], and included 4 hypervariable VNTR loci (VNTR3820, 1982, 3232, and 4120) that were in a consensus loci set for study recent transmission [14, 15]. We used BioNumerics software version 5.0 (Applied Maths, Belgium) to analyze the genotyping data. Tuberculosis cases whose *M. tuberculosis* strains had an identical genotypic pattern were considered a cluster, indicating recent transmission. Cases with a unique genotype pattern indicated reactivation of latent tuberculosis [2]. We restricted the cluster analyses within the local study population in each respective site. Cross-contamination may have occurred if ≥2 isolates from different patients in the same region were processed on the same day in the laboratory and shared the same genotype.

Data Collection

At each field site, trained study workers recruited patients with tuberculosis; obtained their written informed consent; enrolled them in the study; and conducted interviews using a standardized questionnaire to collect information on demographic and clinical characteristics, medical history, and lifestyle behaviors. A diagnostic delay was defined as the time between a patient's report of symptom onset and the date of confirmed diagnosis. Tuberculosis patients had a prior tuberculosis history if they were previously diagnosed and treated for ≥30 days. The questions were translated into the local language as needed. Additional interviews of clustered patients were conducted to identify the contacts, places, and behaviors that were potentially associated with transmission of *M. tuberculosis* between tuberculosis patients in the same cluster.

Calculation of Relative Transmission Rates

Based on the date of collection of the first culture-positive sputum sample, we ordered the tuberculosis patients in a cluster chronologically and estimated the median time between successive cases in a cluster. In each cluster, the index case was defined as the first patient, and all the other patients were secondary cases. A smear-negative transmission event was defined as any secondary case in a cluster that was preceded by a smear-negative tuberculosis case (ie, transmission from a patient with smear-negative tuberculosis). Similarly, all cases that occurred after a patient having a smear-positive result were attributed to transmission by a sputum smear–positive tuberculosis case (ie, transmission from a patient with smear-positive tuberculosis) [16–18]. The minimum relative transmission rate of sputum smear–negative vs sputum smear–positive tuberculosis was calculated as follows: ([the number of smear-negative transmission events]/[the total number of patients with smear-negative tuberculosis]) divided by ([the number of smear-positive transmission events]/[the total number of patients with smear-positive tuberculosis]) [16–18]. We also estimated the minimum relative transmission rate of drug-resistant tuberculosis and MDR tuberculosis vs drug-susceptible tuberculosis isolates.

Statistical Analyses

Univariate analyses compared each potential risk factor in the clustered and nonclustered tuberculosis patients by the Pearson χ^2 test of proportions or the Fisher exact test, as appropriate. To identify the factors that were independently associated with the outcome of tuberculosis transmission, we performed a multiple logistic regression analysis. We used a forward stepwise approach to add covariates to the model. All factors with biological plausibility and $P \leq .2$ in the univariate analysis were considered in the multiple regression models. We also tested for significant interaction terms. We used the Hosmer–Lemeshow test to estimate the goodness of fit of the logistic regression model. A P value <.05 was considered significant. We used Stata software version 13.1/SE (StataCorp, College Station, Texas) for data analyses.

RESULTS

Characteristics of the Study Patients and Strains

During the study period, there were 18 905 cases of suspected tuberculosis in the 5 field sites, of which 2430 (12.9%) were culture-confirmed pulmonary tuberculosis. One isolate per patient was sent to Fudan University for mycobacterial species identification and genotyping. One hundred fifty-six patients were excluded because their isolates were missing or had nontuberculous mycobacteria, because DNA extraction failed, or because cross-contamination likely occurred during laboratory processing (Figure 1). Of the remaining 2366 patients, 2274 (96%) had genotypes available for analysis and 2244 (98.7%) had drug susceptibility test results for INH and RIF. There were 13.5% (303/2244) whose isolate had resistance to INH and/or RIF, and 6.0% (135/2244) had MDR tuberculosis (Table 1). Of the MDR tuberculosis cases, 58.5% (78/130) were new tuberculosis cases (Table 1).

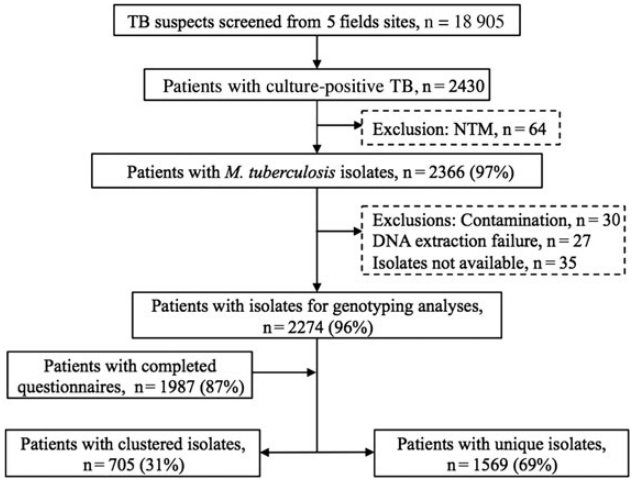

Figure 1. Study population and classification of *Mycobacterium tuberculosis* isolates. Abbreviations: NTM, nontuberculous mycobacteria; TB, tuberculosis.

Estimation of Recent Transmission by Genotyping Analysis

By genotyping analysis, 705 of 2274 (31.0%) patients were identified in 287 clusters (Table 2). The cluster size ranged from 2 (225 clusters) to 13 (1 cluster). The proportion of clustered tuberculosis cases in different sites ranged from 21.7% to 36.1% (Table 2). Among patients with MDR tuberculosis, 43.7% (59/135) were in clusters.

Epidemiological Links of Clustered Patients

Among 614 of the 705 (87%) clustered patients who were investigated, 164 (26.7%) patients from 70 clusters had confirmed or probable epidemiologic links to another patient (Table 2). Of these 164 patients, 10 (6.1%) from 5 clusters were family members (confirmed links), and 55 (33.5%) from 26 clusters lived in the same neighborhood, on the same street, or in the same residential community. The remaining 99 patients (60.4%) from 39 clusters had probable links to another patient, including shared locations where they spent time (eg, underground plaza, senior citizen activity center, the same work camp, internet cafes) or lived in the same village.

Factors Associated With Clustering

A total of 1975 (87%) patients completed a questionnaire from the standardized interviews. Patients who were not interviewed were just as likely to be in a cluster as patients who completed the standardized interview questionnaire (30.0% vs 31.1%, respectively; $P = .82$). By univariate analysis, clustering was not

Table 1. Demographic and Clinical Characteristics of Tuberculosis Patients by Study Site—China, 2009–2012

Characteristic	Pingguo (Guangxi)	Wushen (Sichuan)	Weishi (Henan)	Songjiang (Shanghai)	Wuchang (Heilongjiang)	Total	*P* Value*
No. of patients	324	414	481	797	258	2274	
Female sex	71 (21.9)	95 (23.0)	113 (23.5)	287 (36.0)	83 (32.2)	649 (28.5)	<.001
Age, y, median (IQR)	44 (30–60)	44 (30–56)	51 (30–69)	32 (24–47)	48 (33–60)	41 (27–58)	<.001
Previous tuberculosis history	50 (16.2)	49 (12.7)	66 (13.8)	77 (9.7)	21 (9.1)	263 (11.9)	.01
Drug-resistant cases[a]							
Isoniazid monoresistant	20 (6.2)	22 (5.4)	19 (4.0)	50 (6.4)	16 (6.3)	127 (5.7)	.46
Rifampin monoresistant	9 (2.8)	10 (2.4)	6 (1.3)	7 (0.9)	9 (3.6)	41 (1.8)	.02
MDR[b]	17 (5.3)	38 (9.3)	29 (6.1)	40 (5.1)	11 (4.3)	135 (6.0)	.03

Data are presented as No. (%) unless otherwise specified.

Abbreviations: IQR, interquartile range; MDR, multidrug resistant.

[a] Drug susceptibility test results were not available for 30 cases (3 from Guangxi Province, 4 from Sichuan Province, 2 from Henan Province, 16 from Shanghai metropolitan area, and 5 from Heilongjiang Province).

[b] Resistant to at least isoniazid and rifampin.

* *P* value for comparisons between sites.

associated with the sociodemographic or clinical characteristics of tuberculosis patients (Table 3). Patients who were infected with a Beijing strain were more likely to be clustered than patients who were infected with a non-Beijing strain (odds ratio [OR], 1.67; 95% confidence interval [CI], 1.34–2.07; $P < .001$). Patients in clusters were also more likely to be infected with an MDR strain (OR, 1.79; 95% CI, 1.26–2.56; $P = .001$).

In the multivariable regression model (Table 4), the odds of being in a cluster were still significantly higher among cases with MDR strains (adjusted OR [aOR], 1.86; 95% CI, 1.25–2.63) and among cases infected with Beijing strains (aOR, 1.56; 95% CI, 1.23–2.96).

Transmission of *M. tuberculosis* by Patients With Sputum Smear–Negative Tuberculosis

We determined the relative transmission rate of tuberculosis transmission events caused by patients with sputum smear–negative tuberculosis. Sixty-one patients with unknown sputum smear status were excluded from the analysis. Of the remaining 2213 patients, 732 (33.1%) were smear negative and 1481 (66.9%) were smear positive. Compared with smear-positive patients, smear-negative patients were more likely to be <25 years old, more likely to be new cases, and less likely to have cough and cavitary disease (Supplementary Table 1). In total, 280 of 287 (97.6%) clusters had a source case with known sputum smear result and 404 of 418 secondary cases had known sputum smear status. For the 280 clusters analyzed, the source case was smear negative in 84 clusters (30.0%), and all patients were smear negative in 30 clusters (10.7%). Secondary cases in clusters with a sputum smear–negative source case were more likely to have sputum smear–negative tuberculosis (OR, 1.73; 95% CI, 1.07–2.78), compared with secondary cases in clusters with a source case with sputum smear–positive tuberculosis (Table 5).

Table 2. Distribution of Genotype Clusters in 5 Study Sites—China, 2009–2012

Study Sites (Province)	Total Cases, No.	Clusters, No.	Clustered Cases[a], No. (%)	Maximum Patients in a Cluster, No.	Clustered Cases With Questionnaire, No.	Clustered Case With Epidemiological Link[b], No. (%)
Guangxi	324	47	117 (36.1)	6	113	46 (40.7)
Sichuan	414	42	90 (21.7)[c]	4	82	14 (17.0)
Henan	481	57	149 (30.9)	7	127	35 (27.6)
Shanghai	797	107	255 (32.0)	7	205	41 (20.0)
Heilongjiang	258	34	94 (36.0)	13	87	28 (32.2)
Total	2274	287	705 (31.0)	13	614	164 (26.7)

[a] Cluster analysis was restricted to the local population in each field site.

[b] Patients in the same cluster had probable or confirmed epidemiological links during intensive investigations.

[c] Only the percentage of clustered cases in Sichuan Province was significantly different from the percentage of clustered cases in the other 4 field sites; $P < .05$ for all pairwise comparisons.

Table 3. Univariate Analysis of Risk Factors for Clustering in 5 Field Sites—China, 2009–2012

Characteristics	Nonclustered No. (%)	Clustered No. (%)	OR (95% CI)	*P* Value
Total	1569 (69.0)	705 (31.0)		
Sex				
Female	463 (29.5)	186 (26.4)	1.00	
Male	1106 (70.5)	519 (73.6)	1.16 (.95–1.42)	.126
Age, y, median (IQR)	41 (26–58)	42 (28–59)	1.00 (.99–1.00)	.394
<25	312 (19.9)	136 (19.3)	1.00	
25–44	567 (36.1)	244 (34.6)	0.98 (.77–1.27)	.920
45–64	402 (25.6)	195 (32.3)	1.11 (.85–1.45)	.428
≥65	288 (18.4)	130 (31.1)	1.03 (.78–1.38)	.813
BMI, kg/m^2				
>18.5	907 (66.6)	399 (65.0)	1.00	
≤18.5	454 (33.4)	215 (35.0)	1.08 (.88–1.32)	.471
Previous tuberculosis history				
No	1345 (88.7)	593 (86.7)	1.00	
Yes	172 (11.3)	91 (13.3)	1.20 (.91–1.57)	.189
Diagnosis delay				
<2 wk	234 (17.7)	107 (18.1)	1.00	
2 wk to <1 mo	279 (21.1)	119 (20.2)	0.93 (.68–1.28)	.994
1 mo to <2 mo	310 (23.4)	151 (25.6)	1.07 (.79–1.44)	.680
≥2 mo	500 (37.8)	213 (36.1)	0.93 (.70–1.23)	.619
Cavitation				
No	779 (58.1)	342 (57.0)	1.00	
Yes	561 (41.9)	258 (43.0)	1.05 (.86–1.27)	.640
Cough				
No	147 (10.8)	51 (8.3)	1.00	
Yes	1213 (89.2)	562 (91.7)	1.33 (.95–1.86)	.089
Diabetes (self-report)				
No	1253 (93.1)	556 (92.4)	1.00	
Yes	93 (6.9)	46 (7.6)	1.11 (.77–1.60)	.562
Sputum smear status, AFB				
Negative	514 (32.8)	218 (30.9)	1.00	
Positive	1013 (64.6)	468 (66.4)	1.08 (.89–1.33)	.384
Unknown	42 (2.7)	19 (2.7)	1.06 (.57–1.92)	.822
Drug resistance profile				
Drug susceptible	1355 (87.2)	586 (84.8)	1.00	
Monodrug resistant	122 (7.9)	46 (7.7)	0.87 (.61–1.24)	.445
MDR[a]	76 (4.9)	59 (8.5)	1.79 (1.26–2.56)	.001
Beijing strain				
No	445 (28.4)	135 (19.2)	1.00	
Yes	1124 (71.6)	570 (80.8)	1.67 (1.34–2.07)	<.001
Alcohol use[b]				
No	884 (65.1)	392 (64.2)	1.00	
Yes	473 (34.9)	219 (35.8)	1.04 (.85–1.27)	.671
Smoking[b]				
No	801 (59.1)	341 (55.8)	1.00	
Yes	555 (40.9)	270 (44.2)	1.14 (.94–1.38)	.175

Abbreviations: AFB, acid-fast bacilli; BMI, body mass index; CI, confidence interval; IQR, interquartile range; MDR, multidrug resistant; OR, odds ratio.

[a] Resistant to at least isoniazid and rifampin.

[b] Drinking alcohol or smoking within 6 months before a diagnosis of pulmonary tuberculosis.

Table 4. Results of Multivariable Logistic Regression Analysis of the Risk Factors Associated With Clustering—China, 2009–2012

Characteristics	Adjusted OR[a] (95% CI)	*P* Value
Drug resistance profile		
Drug susceptible	1.00	
Monodrug resistant[b]	0.90 (.63–1.30)	.582
MDR[c]	1.86 (1.25–2.63)	.001
Beijing strain		
No	1.00	
Yes	1.56 (1.23–2.96)	<.001

Abbreviations: CI, confidence interval; MDR, multidrug resistant; OR, odds ratio.

[a] Adjusted for field sites, Beijing strain, and drug resistance profile.

[b] Monoresistant to isoniazid or rifampin.

[c] Resistant to at least isoniazid and rifampin.

Among the 418 secondary cases, we identified 405 cases with known defined smear status of transmission source. One hundred twenty-four of the 405 (30.6%) secondary tuberculosis cases were attributed to transmission from a sputum smear–negative tuberculosis patient, and 281 (69.4%) were attributed to transmission from a sputum smear–positive tuberculosis patient. The minimum number of secondary cases occurring chronologically after a sputum smear–negative tuberculosis patient was 124, for a total of 732 sputum smear–negative tuberculosis cases (16.9%). Similarly, the minimum number of secondary cases occurring chronologically after a sputum smear–positive tuberculosis patient was 281, for a total of 1481 sputum smear–positive tuberculosis patients (18.9%). Thus, the minimum relative transmission rate of sputum smear–negative tuberculosis was 0.89 (95% CI, .68–1.10). To determine whether the order of the first 2 cases in a cluster mattered, we sequentially removed clusters in which the first 2 patients were diagnosed temporally within 0–180 days of each other. The results did not alter the estimate of the minimum proportion of people infected by sputum smear–negative individuals (Supplementary Figure 2).

Transmission of *M. tuberculosis* by Patients With MDR Tuberculosis

By a similar estimation method, 286 source cases and 407 secondary cases had known drug susceptibility test results. The numbers of secondary cases attributed to infection from drug-resistant and MDR tuberculosis among secondary cases were 65 (16.0%) and 36 (8.8%), respectively. The minimum relative transmission rate of MDR tuberculosis cases (26.7% [36/135]) compared with drug-susceptible cases (17.6% [342/1941]) was 1.51 (95% CI, 1.00–2.24). Additionally, 91.7% (33/36) MDR tuberculosis secondary cases were in the 23 clusters that had an index case of MDR tuberculosis, including 8 clusters with MDR tuberculosis cases only (cluster size, 2–7). Furthermore, 17% (11/65) of the drug-resistant tuberculosis cases and 20% (7/36) of the MDR tuberculosis cases were secondary cases linked to a sputum smear–negative source case (Table 5).

DISCUSSION

We present the results of a large-scale, population-based study of the molecular epidemiology of tuberculosis in China, a country with the second-highest tuberculosis burden in the world. At least 1 of every 3 tuberculosis patients was a secondary case due to recent transmission. Patients with sputum smear–negative, culture-positive pulmonary tuberculosis were just as likely to generate secondary tuberculosis cases as patients with sputum smear–positive, culture-positive pulmonary tuberculosis. Patients with MDR tuberculosis were more likely to generate new secondary cases, compared with patients with drug-susceptible tuberculosis.

The proportion of patients in genotypic clusters in this study (31%) was lower than the proportions reported in other studies from low- and high-prevalence countries and regions [1–4, 6, 19–21]. For example, 37% of the tuberculosis cases in Maryland during 1996–2000 [19], 50% of the tuberculosis cases in a gold mining community in South Africa in 1995 [6], and 72% of the tuberculosis cases reported in Malawi during 1995 to 2003 were in genotype clusters [20]. However, a study's sampling frame, study length, and case detection methods impact the number and proportion of clustered cases [22]. In addition, the populations in other studies were restricted to specific subgroups at high risk of tuberculosis, such as gold miners [6] and individuals with human immunodeficiency virus infection [4], homelessness, and drug abuse [19]. Considering the large number of tuberculosis patients in China, with 1.4 million prevalent tuberculosis cases and 1.0 million incident tuberculosis cases annually [7], each serving as a potential source for new infections, the magnitude of potential transmission is staggering.

Consistent with previous findings, we demonstrated that tuberculosis patients who were infected with a Beijing strain were more likely to be in a genotype cluster than tuberculosis patients who were infected with a non-Beijing strain [23]. It has been suggested that Beijing strains have an increased ability to transmit infection, with Beijing strains progressing more rapidly to active tuberculosis [24–26], and they are the most prevalent strain family of *M. tuberculosis* in China. Of interest was the lack of association between the proportion of Beijing strains and the clustered patients in different settings, which may indicate that the transmission of tuberculosis is not solely driven by the endemic-specific *M. tuberculosis* strains (ie, Beijing strain), but rather is the result of complex combination of clinical, social, and bacterial factors. Meanwhile, Beijing strains are a heterogeneous group, and biological hypotheses for their fitness advantages in transmission or pathogenicity warrant further study [23].

Table 5. Characteristics of Secondary Cases in Clusters

Characteristics	Sputum Smear Status of the Index Patient			
	Positive (n = 196)	Negative (n = 84)	Unknown (n = 7)	OR (95% CI)[a]
Smear status of secondary case				
Positive	200 (68.0)	66 (56.4)	4 (57.1)	1.00
Negative	84 (28.6)	48 (41.0)	2 (28.6)	1.73 (1.07–2.78)[b]
Unknown	10 (3.4)	3 (2.6)	1 (14.3)	0.91 (.16–3.67)
Sex				
Female	70 (23.8)	29 (24.8)	0 (0)	1.00
Male	224 (76.2)	88 (75.2)	7 (100.0)	0.94 (.56–1.62)
Age, y				
<25	57 (19.4)	28 (23.9)	2 (28.6)	1.00
25–44	97 (33.0)	41 (35.0)	1 (14.3)	0.86 (.48–1.54)
45–64	92 (31.3)	27 (23.1)	1 (14.3)	0.60 (.32–1.12)
≥65	48 (16.3)	21 (18.0)	3 (42.9)	0.89 (.45–1.77)
Previous tuberculosis history				
No	243 (82.7)	104 (88.9)	5 (71.4)	1.99 (.91–4.83)
Yes	42 (14.3)	9 (7.7)	2 (28.6)	1.00
Unknown	9 (3.1)	4 (3.4)	0 (0)	2.07 (.38–9.66)
Diagnosis delay				
<1 mo	95 (32.3)	43 (36.8)	1 (14.3)	0.90 (.66–1.22)
1 to <2 mo	58 (19.7)	26 (22.2)	1 (14.3)	1.04 (.77–1.37)
≥2 mo	92 (31.3)	26 (22.2)	2 (28.5)	0.56 (.42–.74)[b]
Unknown	49 (15.6)	22 (18.8)	3 (42.9)	0.65 (.36–1.19)
Cavitation				
No	137 (46.6)	64 (54.7)	1 (14.2)	1.00
Yes	109 (37.1)	33 (28.2)	3 (42.9)	0.65 (.38–1.08)
Unknown	48 (16.3)	20 (17.1)	3 (42.9)	0.89 (.46–1.68)
Cough				
No	22 (7.5)	13 (11.1)	0 (0)	1.00
Yes	230 (78.2)	87 (74.4)	4 (57.1)	0.64 (.29–1.45)
Unknown	42 (14.3)	17 (14.5)	3 (42.9)	0.68 (.26–1.84)
Drug resistance profiles (n = 407)[c]				
Drug susceptible	234 (81.5)	103 (90.4)	5 (83.3)	1.00
Drug resistant	53 (18.5)	11 (9.6)	1 (16.7)	0.47 (.21–.96)[b]
MDR[d]	28 (9.8)	7 (6.1)	1 (16.7)	0.56 (.20–1.38)

Data are presented as No. (%) of secondary cases unless otherwise specified.

Abbreviations: CI, confidence interval; MDR, multidrug resistant; OR, odds ratio.

[a] The univariate ORs were calculated for patients in clusters with smear-negative index case vs patients in clusters with smear-positive index case.

[b] $P < .05$.

[c] Drug resistance including drug resistant to isoniazid or/and rifampin.

[d] Resistant to at least isoniazid and rifampin.

The strongest risk factor for clustering was MDR tuberculosis, which emphasizes the ongoing transmission of MDR tuberculosis in the community. More than half of the MDR tuberculosis patients in our study were new tuberculosis cases, evidence that MDR tuberculosis was transmitted person-to-person. A similar finding was reported from the national survey of drug-resistant tuberculosis in China, and transmission of MDR tuberculosis was also observed in other countries [27–29]. There are estimates that <10% of the MDR tuberculosis cases in China were diagnosed, potentially prolonging the infectious period and increasing the opportunities to transmit MDR tuberculosis in the community [30]. Mathematical modeling showed that due to the transmission of MDR tuberculosis, the incidence of MDR tuberculosis in China would increase significantly by 2050 if solely based on current control strategies [31]. It is crucial to address the threat of MDR tuberculosis transmission in China.

In our study, 30.6% of the secondary cases had a putative source case that was a sputum smear–negative tuberculosis patient. Although it has been suggested that the transmission of *M. tuberculosis* was associated with the grade of sputum smear positivity, the relative transmission rate of sputum smear–negative patients, compared with sputum smear–positive patients (0.89), is much higher than that reported in developed countries (0.22–0.24) [11, 16–18, 32]. It is possible that the diagnostic methods and the process used in China (eg, traditional AFB microscopic testing) missed many smear-positive patients, leading to a high proportion of "false" smear-negative patients and, thus, the high relative transmission rate [33]. Most tuberculosis laboratories in China, particularly in rural regions, use sputum smear examinations without culture. They do not capture tuberculosis patients who are sputum smear negative but culture positive, leading to delayed diagnosis and treatment, a longer period of infectiousness, and ongoing transmission of *M. tuberculosis*.

Our study has several limitations. First, patients with unique strains might be misclassified, due to the time period of the study [34]. Second, a sampling bias occurred if the case finding and detection rate in the 5 study sites varied and not all of the truly culture-positive tuberculosis cases were detected. Finally, not all of the tuberculosis patients completed the standardized interview to collect data, making it difficult to identify risk factors for clustering and epidemiological links between tuberculosis patients in the same cluster. Taken together, we likely underestimated the true magnitude and relative rates of transmission.

In conclusion, we present evidence that a considerable proportion of tuberculosis cases in China, including MDR tuberculosis cases, was due to recent transmission of *M. tuberculosis*. Interventions that can effectively reduce the transmission of *M. tuberculosis* include intensified case finding, rapid diagnostic tools with bacteriological confirmation, and appropriate treatment regimens. Additional interventions, such as isolation of infectious tuberculosis cases, screening for latent tuberculosis among contacts, and provision of preventive therapy, might also reduce the incidence of tuberculosis.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. C. Y., X. L., X. S., J. M., and Q. G. designed the study. C. Y., X. S., J. X., F. Li, D. L., F. Liu, J. M., and Q. G. implemented the study. C. Y., T. L., G. S., and X. L. participated in data management. C. Y., X. S., Y. P., R. L., Y. Z., B. L., J. W., X. G., J. H., K. Q., and M. S. participated in data collection. C. Y., G. S., and K. D. performed the data analysis. C. Y. and K. D. wrote preliminary drafts of the report and collected revisions from all of the authors. All authors revised and approved the final manuscript. The corresponding author(s) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Potential conflicts of interest. All authors: No reported conflicts.

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