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Clinical and bacteriological characteristics associated with clustering of multidrug-resistant tuberculosis

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SUMMARY

SETTING—The impact of the genetic characteristics of *Mycobacterium tuberculosis* on the clustering of multi-drug-resistant tuberculosis (MDR-TB) has not been analyzed together with clinical and demographic characteristics.

OBJECTIVE—To determine factors associated with genotypic clustering of MDR-TB in a community-based study.

DESIGN—We measured the proportion of clustered cases among MDR-TB patients and determined the impact of clinical and demographic characteristics and that of three *M. tuberculosis* genetic characteristics: lineage, drug resistance-associated mutations, and *rpoA* and *rpoC* compensatory mutations.

RESULTS—Of 174 patients from California and Texas included in the study, the number infected by East-Asian, Euro-American, Indo-Oceanic and East-African-Indian *M. tuberculosis* lineages were respectively 70 (40.2%), 69 (39.7%), 33 (19.0%) and 2 (1.1%). The most common mutations associated with isoniazid and rifampin resistance were respectively *katG* S315T and *rpoB* S531L. Potential compensatory mutations in *rpoA* and *rpoC* were found in 35 isolates (20.1%). Hispanic ethnicity (OR 26.50, 95%CI 3.73–386.80), infection with an East-Asian *M. tuberculosis* lineage (OR 30.00, 95%CI 4.20–462.40) and *rpoB* mutation S531L (OR 4.03, 95%CI 1.05–23.10) were independent factors associated with genotypic clustering.

CONCLUSION—Among the bacterial factors studied, East-Asian lineage and *rpoB* S531L mutation were independently associated with genotypic clustering, suggesting that bacterial factors have an impact on the ability of *M. tuberculosis* to cause secondary cases.

Keywords

community-based study; transmission; molecular epidemiology

Studies have shown that patients with tuberculosis (TB) are heterogeneous when transmitting *Mycobacterium tuberculosis*.¹ Most of this variability has been attributed to features of the index patient, such as the number of organisms expelled on coughing.² However, some studies have suggested that bacterial characteristics have an impact on the transmission of *M. tuberculosis* and on its pathogenicity, i.e., its ability to cause secondary cases of TB. In one study, organisms of the East-Asian lineage, including the Beijing family, were five times more likely to cause secondary cases than patients with other *M. tuberculosis* lineages.³ In other studies, patients with drug-resistant *M. tuberculosis* were less likely to cause secondary cases than susceptible *M. tuberculosis*.^{4,5} Although some studies have shown no differences,⁶ other studies have suggested that isoniazid (INH) resistant *M. tuberculosis* with mutations in the *inhA* promoter or with S315T *katG* mutations were more likely to be transmitted than those without these mutations.^{7,8} Furthermore, in vitro fitness studies demonstrated that resistant *M. tuberculosis* strains with the most common rifampin (RMP) resistance-associated mutation, *rpoB* S531L, were more fit than strains with less frequent mutations, such as *rpoB* H526Y.⁹ Interestingly, mutations in *rpoA* and *rpoC* have been observed at high frequency in RMP-resistant *M. tuberculosis*, and are considered to be compensatory mutations for any fitness loss that could be caused by the RMP resistance-associated mutation.⁹ It should be noted that the frequency of strains with these compensatory mutations was high in regions with a high burden of multidrug-resistant TB (MDR-TB; i.e., TB resistant to at least INH and RMP),¹⁰ and more frequent among RMP-resistant *M. tuberculosis* isolates that caused secondary cases.^{11,12} However, these studies did not control for other factors known to be associated with transmission.

In this report, we describe the demographic, clinical and bacterial factors associated with genotypic clustering in MDR-TB cases. Genotypic clustering has been used as an indicator of *M. tuberculosis* involved in chains of transmission. We include an analysis of three bacterial characteristics: *M. tuberculosis* lineage, drug resistance-associated mutations, and presence of compensatory mutations in *rpoA* and *rpoC*.

STUDY POPULATION AND METHODS

Study population and data sources

We included all patients with pulmonary TB caused by MDR *M. tuberculosis* organisms (defined by phenotypic drug susceptibility testing) identified between January 2005 and December 2011 from eight health jurisdictions in California (Sacramento, San Mateo, Contra Costa, Alameda, San Diego, Santa Clara, San Francisco and Orange counties), and in five jurisdictions in Texas (Harris, Dallas, Tarrant, Hidalgo and Cameron counties). Some of these patients were enrolled in a parent study to investigate MDR-TB transmission in the United States.¹³ The Human Research Protection Program of the University of California, San Francisco (UCSF), CA, USA, of the Centers for Disease Control and Prevention (CDC) and of each participating institution approved the study protocol.

Data on demographic and clinical characteristics and the epidemiologic links between patients were collected as part of the standard of care and the MDR-TB transmission study.¹³ Lineage of the *M. tuberculosis* isolates was described according to the phylogenetic characterization methodologies previously reported.¹⁴ INH resistance-associated mutations in *katG*, the *inhA* promoter and the RMP resistance-associated mutations in the RMP-resistance determining region (RRDR) of *rpoB* were identified using pyrosequencing.¹⁵ Only amino acid positions 1–191 of *rpoA* (Rv3457c) and positions 245–560 of *rpoC* (Rv0668) (hotspots for possible compensatory mutations¹⁰) were sequenced using *rpoA*, primers (F5 'GGACGTCGAAAGGAAGAAGA3' and R5'GTCTCCACGTCCAGGATCAG3') and *rpoC* primers (F5'CGAAAACCTCTACCGCGAAC3' and R5'GCGACAGGATGTTGTTGGAG3'), respectively.¹¹ Polymerase chain reaction products were sequenced using an ABI377 automatic DNA sequencer (Perkin Elmer, Applied Biosystems, Carlsbad, CA, USA) at the UCSF genomics core facility. Sequence polymorphisms were identified by comparing the consensus sequence of each isolate to the corresponding gene sequence of the H37Rv genome using the A Plasmid Editor, V2.0.46 (W Davis, University of Utah, Salt Lake City, UT, USA).

All isolates were genotyped using spoligotyping and 24-locus mycobacterial interspersed repetitive units (MIRU) typing, as part of the CDC National Tuberculosis Genotyping Service surveillance system,¹⁶ and with insertion sequence (IS) *6110* restriction fragment length polymorphism (RFLP) using standardized methods.¹⁷ Genotypic clustering was defined as two or more MDR-TB isolates from patients from the same state with identical spoligo-type, 24-MIRU type, IS *6110*-RFLP and known drug resistance-associated mutations. Compensatory mutations in *rpoA* and *rpoC* were not included for the definition of clustering. We assumed that patients with clustered MDR-TB isolates within each state had TB due to recent transmission and were part of a chain of transmission. Patients with unique genotypes were considered as having TB due to the reactivation of latent infection. We used the '*n*-1' method to calculate the transmission index that measures the average number of subsequent cases produced by potential index cases.¹⁸

Statistical analysis

We described the demographic, clinical and bacterial characteristics of *M. tuberculosis* isolates associated with *M. tuberculosis* genetic clustering (the outcome) using logistic regression and exact logistic regression where expected cell counts were <5. We performed full-fitted exact logistic regression models, first including variables with $P < 0.25$ in the unadjusted analysis, then including those with $P < 0.20$. Correlated variables were removed from the model to identify the most stable, parsimonious, and informative model possible. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences, version 18.0, IBM Corp, Armonk, NY, USA) and SAS (version 9.4, SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient characteristics

During the study period, 169 patients from California and 38 from Texas had pulmonary TB caused by MDR organisms, including four with XDR-TB (i.e., MDR-TB with additional resistance to fluoroquinolones and second-line injectable drugs) and 29 pre-XDR-TB (MDR-TB with resistance to either fluoroquinolones or second-line injectable drugs). After excluding cases without *M. tuberculosis* DNA or clinical data, 140 patients (83%) from California and 34 (89%) from Texas were included in the analysis. The characteristics of included vs. excluded patients are shown in Table 1. The mean age of the 174 patients included was 39.1 (± 16) years, 80 (46%) were female and most were Asian ($n = 104$, 59.8%) or White ($n = 63$, 36.2%) (Table 2).

Genetic characteristics of *M. tuberculosis*

Of the 174 patients, the number infected by East-Asian, Euro-American, Indo-Oceanic and East-African-Indian lineages were respectively 70 (40.2%), 69 (39.7%), 33 (19%) and 2 (1.1%) (Table 2). The INH resistance-associated mutation, *katG* S315T, and mutations in the *inhA* promoter were observed in respectively 124 (71.3%) and 40 (23%) of the isolates. The remaining 10 isolates did not have mutations in these genes, and none had mutations in both genes. The RMP resistance-associated mutation *rpoB* S531L ($n = 105$, 60.3%) (Table 2) was the most frequent mutation, followed by H526D ($n = 18$, 10.3%) and H526Y ($n = 12$, 6.9%). Five patients had isolates with two mutations each in *rpoB* (Table 2 footnote). *M. tuberculosis* from six patients did not have a mutation in *rpoB* RRDR.

We identified four unique single nucleotide polymorphisms (SNPs) in *rpoA* and 17 unique SNPs in *rpoC*, of which we excluded two from the analysis: A542A (1626 C→G), associated with Euro-American lineage but not with resistance,^{10,11} and the synonymous mutation R480R (1440 C→T). These SNPs were observed in respectively 23 and 2 patients. The 19 non-synonymous potentially compensatory mutations were found in 35 *M. tuberculosis* isolates (35/174, 20.1%) (Table 3). Five isolates had two mutations each in the *rpoC* gene. Twelve unique isolates had *rpoC* mutations in codon 483 (V483G, $n = 9$ and V483A, $n = 3$) and six had the A466V mutation. The mutation P434V in *rpoC* was associated with two haplotypes (1300 C→G and 1301 C→T), and occurred in the same isolate (Table 3). The A466V and D271G mutations have not been reported previously.

There were four different *rpoA* mutations in four isolates (4/174, 2.3%), two of which had not been reported before (G115A, T127A) (Table 3).

The frequency of *M. tuberculosis* isolates with *rpoA* or *rpoC* mutations varied depending on the *rpoB* mutation: they were more frequent in isolates with the S531L mutation (82.9% vs. 17.2%, odds ratio [OR] 4.01, 95% confidence interval [CI] 1.56–10.03) (Table 2). The proportion of *M. tuberculosis* isolates with *rpoA* and *rpoC* mutations was different among the different lineages, although this difference was not statistically significant (Table 2).

Genotypic clustering

Of the 174 *M. tuberculosis* isolates, 23 (13.2%) were placed in eight genotypic clusters (Table 4). Five clusters were composed of East-Asian lineage isolates and three of Euro-American lineage isolates. The transmission indices in East-Asian and Euro-American lineage were respectively 0.143 and 0.072. Isolates in two clusters had different *rpoC* mutations. In cluster 1, the patient with the F452L mutation was the earliest case in the cluster (December 2005) based on the date of diagnosis. The remaining two patients were diagnosed in December 2007 (G332T mutation) and August 2008 (F452L mutation). In cluster 7, the patient with the double mutation was reported in January 2007 and the patient with the wild-type *rpoA* and *rpoC* in May 2009. Patients in cluster 7 reported knowing each other. The only other epidemiologic link reported was among two of the four patients in cluster 3.

In the unadjusted analysis (Table 5), Hispanics (OR 2.72, 95%CI 1.11–6.62), patients with excessive alcohol consumption (OR 6.61, 95%CI 2.11–20.50), and those infected with an East-Asian lineage (OR 3.27, 95%CI 1.30–8.21) were more likely to be in genotypic clusters. The adjusted analysis using all values with $P < 0.25$ was unstable due to the small number of outcomes (cluster). The more parsimonious model using values with $P < 0.20$ was unstable due to interactions between excessive alcohol use, Asian race, and East-Asian lineage. Alcohol was reported in one Asian patient, and Asian race was correlated with East-Asian lineage; we therefore removed alcohol and race from the adjusted model. The most stable and parsimonious model showed that Hispanic ethnicity (OR 26.5, 95%CI 3.73–386.80), infection with an East-Asian *M. tuberculosis* lineage (OR 30.0, 95%CI 4.20–462.40) and the presence of an *rpoB* S531L mutation (OR 4.03, 95%CI 1.05–23.10) were independently associated with genotypic clustering (Table 5). To explore the role of excessive alcohol use, we performed a similar analysis, stratified by race, and found that among non-Asians, excessive alcohol was associated with genotypic clustering (Table 6).

DISCUSSION

In this study of the clustering of patients with TB caused by MDR *M. tuberculosis*, we found that Hispanic ethnicity, being infected with an *M. tuberculosis* strain from the East-Asian lineage and with an *rpoB* S531L mutation were independent risk factors for genotypic clustering of TB cases. Compensatory mutations in *rpoA* and *rpoC* were not associated with clustering. To our knowledge, this is the first study to include a systematic analysis of clinical and epidemiologic data together with drug resistance-associated mutations,

compensatory mutations and *M. tuberculosis* lineage on transmission and pathogenesis (measured by clustering) of MDR-TB.

The distribution of the mutations causing INH and RMP resistance was similar to that noted in previous reports.^{23,24} The frequency of potential compensatory mutations in *rpoA* and *rpoC* mutations was also similar to that in other reports.^{10–12,22} In all studies, *rpoC* mutations were more frequent than *rpoA* mutations. Although most of the *rpoA* and *rpoC* mutations have been reported previously,^{10–12,19–22} we identified four new mutations (G115A and T127A in *rpoA* and D271G and A466V in *rpoC*) in the hotspot area that potentially affect the interaction between the *rpoA*, *rpoB* and *rpoC* subunits of the RNA polymerase, and which have not been previously reported.¹⁰

The cluster rate in our study population was 13.8%, and was independently associated with being infected with *M. tuberculosis* strains from the East-Asian lineage. The East-Asian *M. tuberculosis* lineage has been associated with clustering in many molecular epidemiologic studies in East Asia;²⁵ however, its impact in areas outside Asia is more controversial.²⁶ In vitro and animal model studies have suggested that the East-Asian lineage is more pathogenic and virulent compared with other strains,²⁷ and the production of phenolic glycolipid has been proposed as a possible mechanism.²⁸ Despite the uncertain pathogenesis, our findings suggest a unique role of the East-Asian lineage in MDR-TB transmission. We also found that *rpoB* S531L was associated with genetic clustering, which supports the findings that this mutation has no fitness cost in in vitro studies,⁹ causing 40–73% of the *M. tuberculosis* RMP resistance,^{24,29,30} and is associated with compensatory mutations in *rpoA* and *rpoC* genes.^{11,22,31}

Contrary to recent reports, we did not find any association between *rpoA* and *rpoC* mutations and clustering. de Vos and Li analyzed convenience samples from South Africa and China, respectively, and found that mutations in *rpoC* were significantly associated with clustering of RMP-resistant *M. tuberculosis*;^{11,12} however, they did not consider other factors known to be associated with genotypic clustering. We did not include the *rpoA* and *rpoC* genotype in the definition of clustering, as the implication for their phenotype and microevolution of *M. tuberculosis* has not been defined for most of the mutations.

Hispanic patients were more likely to be in a genotypic cluster. This result is similar to the cross-sectional study that evaluated the transmission of MDR-TB in the United States.¹³ Excessive alcohol use was found to be an independent risk factor associated with genotypic clustering in non-Asian patients. As it was only reported in one Asian patient, we could not evaluate the impact of alcohol consumption in this population. Based on the latest results from the 2010 National Survey on Drug Use and Health, excessive alcohol use is rare among Asian populations.³² Patients with chronic use of alcohol are known to be lymphopenic, with a reduced response to mitogen stimulation and impaired delayed-type hypersensitivity responses.³³ Excessive use of alcohol, usually in conjunction with homelessness, injection drug use, and smoking, has been widely reported as an important risk factor for TB transmission in molecular epidemiologic studies, especially in low TB incidence areas.³⁴

The present study had several limitations. First, our sample size was small and, as not all MDR-TB isolates from the study period were included, it is likely that we underestimated the number of genotypic clustered cases. Second, as both Indo-Oceanic and East-African-Indian lineages were underrepresented among the isolates studied, no conclusions can be drawn about their potential for clustering. Third, some factors that have been shown to be associated with transmission, such as the presence of live *M. tuberculosis* in cough droplets,² delay in treatment initiation, socio-economic status, or place of exposure, were not measured in our study. Fourth, only partial regions of *rpoA* and *rpoC* were sequenced for mutation detection. However, the sequenced area includes the *rpoA-rpoC* interaction region of the *rpoC* gene, which has the potential to ameliorate the fitness cost of *rpoB* resistance mutations.¹⁰ Moreover, most of the mutations found outside of the *rpoA-rpoC* interaction region have not shown convergent evolution as has been observed for drug resistance mutations³⁵ and for compensatory mutations,¹⁰ and the likelihood that they are compensatory mutations is therefore lower. Finally, we were not able to perform whole genome sequencing, which has been shown to better delineate the transmission links.

CONCLUSIONS

This community-based study presents a systematic analysis of clinical, epidemiologic and bacterial genetic factors associated with clustering of MDR-TB in two US states. We found that *M. tuberculosis* from the East-Asian lineage and isolates with the *rpoB* S531L mutation are bacterial factors independently associated with genetic clustering, suggesting that bacterial factors may have an impact on the ability of *M. tuberculosis* to cause secondary cases. In addition, we found that Hispanic patients were more likely to be part of a genetic cluster, as were non-Asian patients with excessive use of alcohol.

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Conflicts of interest: none declared.

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Table 1

Clinical characteristics of included and excluded patients

	Included (<i>n</i> = 174)	Excluded (<i>n</i> = 33)	<i>P</i> value
	<i>n</i> (%)	<i>n</i> (%)	
Age, years, mean ± SD	39.1 ± 16	37.7 ± 16.6	0.646
Female sex	80 (46)	16 (48.5)	0.881
Race *			0.497
Asian	104 (59.8)	20 (60.6)	
White	63 (36.2)	11 (33.3)	
African American	4 (2.3)	2 (6.1)	
Unknown	3 (1.7)	0	
Hispanic	55 (31.6)	7 (21.2)	0.225
Prior anti-tuberculosis treatment	46 (26.4)	12 (36.4)	0.325
HIV infection *	5 (2.9)	0	0.380
Diabetes mellitus *	7 (4)	1 (3)	0.786
BCG vaccination	33 (19)	5 (15.2)	0.604
Homeless in last 12 months *	7 (4)	0	0.602
Correctional facility at time of diagnosis *	5 (2.9)	3 (9.1)	0.113
Long-term facility at time of diagnosis *	2 (1.1)	1 (3)	0.392
Injecting drug user in last 12 months *	2 (1.1)	0	1.000
Excessive alcohol use in last 12 months *	22 (12.6)	4 (12.1)	1.000

* As expected cell counts were <5, *P* value was calculated using Fisher's exact test.

SD = standard deviation; HIV = human immunodeficiency virus; BCG = bacille Calmette-Guérin.

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Table 2

Clinical and microbiological characteristics of patients with and without *M. tuberculosis* *rhoA* or *rhoC* mutations and ORs of having mutations in *rhoA* and *rhoC* genes

	<i>rhoA</i> or <i>rhoC</i> mutations				OR (95%CI)	P value
	All (n = 174)	Yes (n = 35)	No (n = 139)			
Age, years, mean ± SD	39.1 ± 16	35.2 ± 13.9	40.1 ± 16.3	0.98 (0.95–1.01)	0.115	
Female sex	80 (46)	22 (62.9)	58 (41.7)	2.25 (1.05–4.83)	0.038	
Race*						
Asian	104 (59.8)	20 (57.1)	84 (60.4)	1.00	—	
White	63 (36.2)	14 (40)	49 (35.3)	1.20 (0.51–2.76)	0.782	
African American	4 (2.3)	1 (2.9)	3 (2.2)	1.40 (0.03–18.50)	1.000	
Unknown	3 (1.7)	0	3 (2.2)	—	—	
Hispanic ethnicity	55 (31.6)	12 (34.3)	43 (30.9)	1.15 (0.53–2.53)	0.723	
Prior anti-tuberculosis treatment	46 (26.4)	10 (28.6)	36 (25.9)	1.16 (0.50–2.69)	0.727	
HIV infection*	5 (2.9)	0	5 (3.6)	—	—	
Diabetes mellitus*	7 (4)	0	7 (5)	—	—	
BCG vaccination	33 (19)	5 (14.3)	28 (20.1)	0.66 (0.24–1.86)	0.432	
Homeless in last 12 months*	7 (4)	1 (2.9)	6 (4.3)	0.65 (0.01–5.68)	1.000	
Correctional facility at time of diagnosis*	5 (2.9)	3 (8.6)	2 (1.4)	6.23 (0.69–77.50)	0.115	
Long-term facility at time of diagnosis*	2 (1.1)	0	2 (1.4)	—	—	
Injecting drug use in last 12 months*	2 (1.1)	1 (2.9)	1 (0.7)	4.05 (0.05–323.40)	0.721	
Excessive alcohol use in last 12 months*	22 (12.6)	4 (11.4)	18 (12.9)	0.91 (0.21–3.04)	1.000	
<i>M. tuberculosis</i> lineages*						
East-Asian	70 (40.2)	12 (34.3)	58 (41.7)	1.00	—	
Euro-American	69 (39.7)	15 (42.9)	54 (38.8)	1.34 (0.53–3.45)	0.638	
Indo-Oceanic	33 (19)	6 (17.1)	27 (19.4)	1.07 (0.30–3.50)	1.000	
East-African Indian	2 (1.1)	2 (5.7)	0	—	—	
Isoniazid resistance-associated mutations in: <i>katG</i> S315T	124 (71.3)	31 (88.6)	93 (66.9)	3.83 (1.28–11.50)	0.016	

	<u><i>rpoA</i> or <i>rpoC</i> mutations</u>				OR (95%CI)	P value
	All (n = 174)	Yes (n = 35)	No (n = 139)			
	n (%)	n (%)	n (%)	n (%)		
<i>inhA</i> promoter [‡]	40 (23)	4 (11.4)	36 (25.9)	0.37 (0.12–1.12)	0.078	
Rifampin resistance-associated mutations						
<i>rpoB</i> S531L	105 (60.3)	29 (82.9)	76 (54.7)	4.01 (1.56–10.30)	0.003	
No <i>rpoB</i> S531L and wt <i>rpoB</i> [§]	69 (39.7)	6 (17.1)	63 (45.3)	1	—	

* As expected cell counts were <5, OR, (95%CI) and P values were calculated using exact logistic regression.

[‡] 10 isolates did not have mutations in *katG* or *inhA*.

[§] 36 isolates had a mutation –15T, 2 isolates –8A, and 2 isolates –17T.

[§] There were six isolates with wt *rpoB* and 63 cases with 68 *rpoB* mutations other than S531L (five patients had two mutations each): H526D (n = 18), H526Y (n = 12), D516V (n = 8), H526R (n = 5), L533P (n = 3) Q513L (n = 2), D516Y (n = 2), N519K (n = 2), H526A (n = 2), H526C (n = 2), H526L (n = 2), H526Q (n = 1), Q510L (n = 1), L511P (n = 1), Q513K (n = 1), D516F (n = 1), S522L (n = 1), S25del (n = 1), H526N (n = 1), K527P (n = 1), H526S (n = 1), H526S (n = 1). Five patients had two mutations and the following combinations: D516V and Q510L, D516Y and 525del, H526N and Q513L, H526Q and L533P, H526S and K527P.

OR = odds ratio; CI = confidence interval; SD = standard deviation; HIV = human immunodeficiency virus; BCG = bacille Calmette-Guérin; wt = wild-type.

Table 3

tpoA and *tpoC* mutations in MDR-TB isolates

Gene	Mutation site	Isolates <i>n</i>	Nucleotide substitution	Amino acid change	References
<i>tpoC</i>	812	1	A→G	D271G	Not reported
<i>tpoC</i>	994	1	G→A	G332S	19, 20
<i>tpoC</i>	1297	1	G→A	G433S	10, 12, 19, 20
<i>tpoC</i>	1300	1	C→G	P434V	21
<i>tpoC</i>	1301	1	C→T	P434V	21
<i>tpoC</i>	1301	1	C→A	P434Q	19, 20
<i>tpoC</i>	1354	3	T→C	F452L	21, 22
<i>tpoC</i>	1397	6	C→T	A466V	Not reported
<i>tpoC</i>	1448	3	T→C	V483A	11, 12, 19–22
<i>tpoC</i>	1448	9	T→G	V483G	10–12, 20, 21
<i>tpoC</i>	1471	3	A→G	I491V	10–12, 19, 20
<i>tpoC</i>	1519	3	T→G	L507V	12, 21
<i>tpoC</i>	1547	1	T→C	L516P	10, 19–21
<i>tpoC</i>	1562	1	C→A	A521D	10, 12, 20
<i>tpoC</i>	1573	1	C→A	H525N	10, 12
<i>tpoA</i>	344	1	G→C	G115A	Not reported
<i>tpoA</i>	379	1	A→G	T127A	Not reported
<i>tpoA</i>	559	1	A→G	T187A	10, 12, 20, 21
<i>tpoA</i>	569	1	A→G	D190G	20, 21

MDR-TB = multidrug-resistant tuberculosis.

Table 4

Clusters identified in MDR-TB isolates from 140 patients from California and 34 from Texas

Clusters	Location	Isolates <i>n</i>	Lineage	<i>rpoA</i> mutations	<i>rpoC</i> mutations	<i>rpoB</i> mutations
1	California	3	East-Asian	None	G332T in 1	S531L
2	California	4	Euro-American	None	F452L in 2	S531L
3	California	4	East-Asian	None	wt in 4	S531L
4	California	4	East-Asian	None	wt in 4	S531L
5	California	2	East-Asian	None	wt in 4	H526D
6	Texas	2	Euro-American	None	wt in 2	S531L
7	Texas	2	Euro-American	None	wt in 2	S531L
8	Texas	2	East-Asian	None	A466V and L507V in 1; wt in 1	H526D
				None	wt in 2	S531L

MDR-TB = multidrug-resistant tuberculosis; wt = wild-type.

Table 5

Clinical and microbiological factors associated with clustering

	Clustering*		Unadjusted		Adjusted	
	Yes (n = 23) n (%)	No (n = 151) n (%)	OR (95%CI)	P value	OR (95%CI)	P value
Age ≥ 65 years [†]	2 (8.7)	9 (6.0)	1.56 (0.15–8.37)	0.846		
Male sex	11 (47.8)	79 (52.3)	0.79 (0.33–1.90)	0.598		
Asian race	11 (47.8)	95 (62.9)	0.54 (0.22–1.31)	0.171		
Hispanic ethnicity	12 (52.2)	43 (28.5)	2.72 (1.11–6.62)	0.028	26.50 (3.73–386.80)	<0.001
Previous anti-tuberculosis treatment	3 (13.0)	43 (28.5)	0.46 (0.13–1.67)	0.239		
HIV infection	0	5 (3.3)	—	—		
Diabetes mellitus [†]	3 (13.0)	4 (2.6)	5.54 (0.76–35.40)	0.095	3.64 (0.35–35.30)	0.355
BCG vaccination [†]	1 (4.3)	32 (21.2)	0.17 (0.004–1.17)	0.087	0.18 (0.004–1.39)	0.140
Homeless in last 12 months [†]	2 (8.7)	5 (3.3)	2.72 (0.24–18.00)	0.473		
Correctional facility at time of diagnosis [†]	1 (4.3)	4 (2.6)	1.68 (0.03–18.00)	1.000		
Long-term care facility at time of diagnosis	0	2 (1.3)	—	—		
Injecting drug user in last 12 months [†]	1 (4.3)	1 (0.7)	6.65 (0.08–535.00)	0.497		
Excessive alcohol in last 12 months [†]	9 (39.1)	13 (8.6)	6.61 (2.11–20.50)	<0.001		
East-Asian lineage infection	15 (65.2)	55 (36.4)	3.27 (1.30–8.21)	0.012	30.00 (4.20–462.40)	<0.001
<i>rpoA</i> or <i>rpoC</i> mutations [†]						
No mutations	19 (82.6)	120 (79.5)	1.00	—		
Overall mutations	4 (17.4)	31 (20.5)	0.80 (0.19–2.66)	0.954		
<i>rpoC</i> mutation	4 (17.4)	27 (17.9)	0.95 (0.22–3.17)	1.000		
<i>rpoA</i> mutation	0	4 (2.7)	—	—		
<i>rpoB</i> mutation status						
Without S531L mutation	6 (26.1)	63 (41.7)	1.00	—		
With S531L mutation	17 (73.9)	88 (58.3)	2.03 (0.76–5.43)	0.159	4.03 (1.05–23.10)	0.040
<i>katG</i> mutation status						
Without <i>katG</i> mutation	4 (17.4)	46 (30.5)	1.00	—		
With <i>katG</i> mutation	19 (82.6)	105 (69.5)	2.08 (0.67–6.46)	0.205		

Defined as isolates collected in the same state and sharing identical spoligotyping, 24-locus mycobacterial interspersed repetitive units typing, insertion sequence *IS6110* restriction fragment length polymorphism pattern and drug-resistance-associated mutations.

⁷As expected cell counts were <5, unadjusted ORs and *P* values were calculated using the exact logistic method.

OR = odds ratio; CI = confidence interval; HIV = human immunodeficiency virus; BCG = bacille Calmette-Guérin.

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Most parsimonious model of clinical and microbiological factors associated with clustering in non-Asian patients

Table 6

	Clustering*		Unadjusted		Adjusted	
	Yes (n = 12) n (%)	No (n = 56) n (%)	OR (95%CI)	P value	OR (95%CI)	P value
Age ≥ 65 years [†]	0	2 (3.7)	Could not calculate			
Hispanic [†]	12 (100)	42 (75.0)	Could not calculate			
Diabetes mellitus [†]	3 (25.0)	1 (1.8)	17.1 (1.23–978.90)	0.031	10.0 (0.53–751.00)	0.169
BCG vaccination [†]	1 (8.3)	10 (17.9)	0.42 (0.01–3.59)	0.751		
Injecting drug user in last 12 months [†]	1 (8.3)	1 (1.8)	4.74 (0.06–392.20)	0.656		
Excessive alcohol in last 12 months [†]	9 (75.0)	12 (22.2)	10.0 (2.09–66.80)	0.001	7.78 (1.55–52.50)	0.009
East-Asian lineage [†]	4 (33.3)	7 (12.5)	3.42 (0.60–17.70)	0.188		
With S531L mutation [†]	6 (50.0)	36 (65.5)	0.53 (0.12–2.29)	0.495		
With <i>kafG</i> mutation [†]	8 (66.7)	40 (72.7)	0.75 (0.17–3.93)	0.918		

* Defined as isolates collected in the same state and sharing identical spoligotyping, 24-locus mycobacterial interspersed repetitive units typing, insertion sequence 6110 restriction fragment length polymorphism pattern and drug-resistance-associated mutations.

[†] As expected cell counts were <5, unadjusted ORs and P values were calculated using the exact logistic method.
OR = odds ratio; CI = confidence interval; BCG = bacille Calmette-Guérin.