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RESEARCH ARTICLE

Characteristics of compensatory mutations in the *rpoC* gene and their association with compensated transmission of *Mycobacterium tuberculosis*

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Abstract **The aim of this study was to characterize *rpoC* gene mutations in *Mycobacterium tuberculosis* (MTB) and investigate the factors associated with *rpoC* mutations and the relation between *rpoC* mutations and tuberculosis (TB) transmission. A total of 245 MTB clinical isolates from patients with TB in six provinces and two municipalities in China were characterized based on gene mutations through DNA sequencing of *rpoC* and *rpoB* genes, phenotyping via standard drug susceptibility testing, and genotypic profiling by mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing. Approximately 36.4% of the rifampin-resistant isolates harbored nonsynonymous mutations in the *rpoC* gene. Twenty-nine nonsynonymous single mutations and three double mutations were identified. The *rpoC* mutations at locus 483 (11.3%) were predominant, and the mutations at V483G, W484G, I491V, L516P, L566R, N698K, and A788E accounted for 54.5% of the total detected mutations. Fifteen new mutations in the *rpoC* gene were identified. Rifampin resistance and *rpoB* mutations at locus 531 were significantly associated with *rpoC* mutations. MIRU-VNTR genotype results indicated that 18.4% of the studied isolates were clustered, and the *rpoC* mutations were not significantly associated with MIRU-VNTR clusters. A large proportion of *rpoC* mutation was observed in the rifampicin-resistant MTB isolates. However, the findings of this study do not support the association of *rpoC* mutation with compensated transmissibility.**

Keywords tuberculosis; drug resistance; compensatory mutations; transmission

Introduction

Tuberculosis (TB), especially drug-resistant TB, poses a serious challenge to disease control in China. An effective TB control program should be based on a comprehensive understanding of the circulating *Mycobacterium tuberculosis* (MTB) in the country. Previous studies have indicated that the recent transmission of MTB strains, including multidrug-resistant (MDR) ones (resistance to at least isoniazid and rifampin), contributes substantially to the TB disease burden in China; about one-third of MDR-TB cases are attributed to this recent transmission [1,2]. The factors that contribute to the development and transmission of MTB are complex, and previous studies have focused on inadequate therapy, poor implementation of national TB programs, and host susceptibility. However, MTB-related characteristics, including the ability to infect the host, persist, proliferate, and transmission have often been ignored. Gene mutations that confer drug resistance are commonly associated with fitness costs [3,4], and drug-resistant bacteria are usually considered to be less fit than susceptible strains and less likely to cause epidemics than drug-sensitive ones. However, a previous study revealed that several drug resistance mutations concomitantly lower or obviate fitness costs and that low average fitness costs of mutations are associated with increased severity of epidemics caused by drug-resistant TB [5]. Mathematical

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model results have also shown that the relative fitness of drug-resistant strains may play an important role in spreading MDR-TB [6,7].

Rifampin (RMP) is an important first-line anti-TB drug, and resistance to RMP poses a serious challenge to the effective treatment of TB or MDR-TB. RMP resistance results from mutations in the RMP resistance-determining region (RRDR) of the *rpoB* gene, which encodes the β subunit of RNA polymerase. RMP resistance can be caused by different mutations in the *rpoB* gene, but clinical strains with *rpoB* mutation S531L are highly prevalent [8]. The success of S531L mutations in clinical MTB isolates may depend not only on a low initial fitness cost but also on the ability to acquire compensatory mutations for further reducing the fitness cost; this compensatory evolution can reduce initial fitness defects caused by particular drug resistance-conferring mutations and is potentially important for the long-term persistence of drug resistance [9–12].

Among fitness compensatory mutations with the *rpoB* gene, *rpoA* and *rpoC* genes, which encode α and β′ subunits of RNA polymerase, respectively, have been extensively investigated; a study on MTB has revealed that mutations in *rpoA* and *rpoC* are common among clinical strains with *rpoB* S531L mutations [13]. Other studies assessed the possible link between *rpoC* mutations and MTB transmission by comparing the proportion of compensatory *rpoC* mutations in clustered or nonclustered strains; these studies found that compensatory mutations are more common in clustered than in nonclustered strains and concluded that *rpoC* mutations may restore the fitness cost caused by resistance-conferring mutations, thereby enhancing the ability of MDR-TB strains to spread [14,15]. However, MDR-TB strains with compensatory mutations are unlikely to cluster and do not promote the influence of compensatory mutations on MDR-TB transmission [16]. These contradicting conclusions highlight the need for future studies. In the current study, we used strains collected from four provinces and two municipalities to describe the profile of mutations in the *rpoC* gene and determine if compensatory mutations facilitate the spreading of MTB.

Materials and methods

Study population

The study sample comprised MTB cultures isolated from patients with smear-positive pulmonary TB who visited local TB hospitals or dispensaries between February 2012 and October 2013 in four provinces (Heilongjiang, Henan, Sichuan, and Zhejiang) and two municipalities (Chongqing and Tianjin) in China. Only one isolate from each patient was included in this study.

Drug susceptibility testing

Acid-fast bacillus (AFB) positive cultures recovered from a Löwenstein–Jensen (L–J) medium were used for identification and drug susceptibility testing (DST). MTB identification was performed by testing susceptibility to p-nitrobenzoic acid. Susceptibility to RMP, isoniazid, streptomycin, ethambutol, kanamycin, and ofloxacin was examined by applying the proportion method in L–J medium in accordance with the World Health Organization's standard proportional method.

DNA extraction

DNA was extracted from MTB isolates grown on L–J medium via a simple method. Bacteria were suspended in 400 μL of 1 × TE buffer, heat killed at 80 °C for 30 min, boiled at 100 °C for 15 min, and centrifuged at 12 000 *g* for 15 min to remove cell debris. The supernatant was stored at −20 °C for further use.

rpoB and *rpoC* gene amplification and sequence analysis

Polymerase chain reaction (PCR) amplification was performed, followed by DNA sequencing of the *rpoB* RMP RRDR region, which is an 81 bp hotspot region (codons 507 to 533) of the *rpoB* gene. The primers for *rpoB* were *rpoB* F(5′-TACGGTCGGCGAGCTGATCC-3′) and *rpoB* R(5′-TACGGCGTTTCGATGAACC-3′). The entire *rpoC* locus (Rv0668) was amplified and sequenced. The primer sequence used for *rpoC* was obtained from a previous work [14]. PCR amplification was performed under the following conditions: 5 min of denaturation at 94 °C, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, elongation at 72 °C for 30 s, and final extension of 7 min at 72 °C. The amplicons were purified and sequenced with the ABI DNA sequencer model 377, and sequences were analyzed using DNAstar and BioEdit software.

Molecular genotyping

MTB Beijing genotype was identified via RD-105 multiplex PCR [17]. Mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing was performed on chromosomal DNA extracted from MTB isolates. PCR was conducted to amplify a standard panel of 15 loci [18], and the PCR products were separated on 2% agarose gel with 50 or 100 bp DNA markers. DNA extracted from the reference strain H37Rv was used as positive control. A MIRU-VNTR cluster was defined when two or more isolates from different patients showed the same 15-locus MIRU-VNTR genotype profiles; otherwise, the isolates were considered unique.

Statistical analyses

The cluster of MIRU-VNTR genotyping was performed with the MIRU-VNTRplus web application (http://www.miru-vntrplus.org/MIRU/index.faces). Statistical analyses were performed with SAS9.1 (SAS Institute Inc.). Univariate and multivariate logistic regression were used to identify the risk factors for *rpoC* mutation or factors for MIRU-VNTR cluster. Variables with $P < 0.05$ were considered statistically significant.

Results

Characteristics of the study population and isolated mycobacteria

A total of 257 patients were included in this study. Among the mycobacterial isolates, eight were identified as nontuberculous mycobacteria. Among the 249 MTB isolates, 245 had available results on DST, genotyping, and gene sequencing. The median age of the study patients was 44.2 ± 16.3 years (14–82 years), and 183 (75.0%) patients were male. Among the 243 patients with available treatment history, 125 (51.4%) were new. According to the DST results, 94 (38.4%) were RMP-sensitive isolates, and 151 (61.6%) were RMP-resistant isolates, among which 103 (42.0%) were RMP monoresistant or MDR isolates and 48 (19.6%) were pre-extensively drug-resistant (pre-XDR, defined as strains resistant to isoniazid and RMP and to at least one fluoroquinolone or one second-line injectable agent [SLI]: kanamycin, amikacin, or capreomycin) or XDR (defined as strains with resistance to at least isoniazid, RMP, a fluoroquinolone, and at least one SLI) isolates. RD-105 multiplex PCR revealed that 198 (80.8%) were Beijing family strains.

Characteristics of mutations in the *rpoC* gene

Gene sequence analyses revealed that 55 (36.4%) RMP-resistant isolates harbored 29 nonsynonymous single mutations and 3 double mutations in the *rpoC* gene. The *rpoC* gene loci frequently involved in the mutations were codon 483 (11.3%), followed by codons 484 (2.0%), 491 (2.0%), 698 (2.0%), 516 (1.3%), 332 (1.3%), 521 (1.3%), 566 (1.3%), and 788 (1.3%). Seven nonsynonymous mutations at V483G, W484G, I491V, L516P, L566R, N698K, and A788E accounted for 54.5% of the total detected mutations. Fifteen new mutations, which have not been previously reported, were found in the *rpoC* gene (Table 1, Fig. 1). For the 94 RMP-susceptible isolates, 4 (4.26%) nonsynonymous (R703C, I800V, D279G, and A466E) and 3 synonymous (2 isolates with P1040P and one isolate with G109G) mutations were identified. The results were confirmed by repeated phenotypic DST and sequencing of the RRDR of *rpoB* and the entire *rpoC* gene.

Factors associated with *rpoC* mutation

Logistic regression models were used to identify the possible risk factors associated with *rpoC* mutation. The analyzed factors included age, gender, treatment history, strain family, RMP susceptibility, and *rpoB* mutation. Univariate and multivariate analyses revealed that host factors, such as age, gender, and treatment history, were not associated with mutations in the *rpoC* gene. The strain background Beijing family was not associated with mutations in *rpoC* either, whereas RMP resistance (monoresistant or MDR, pre-XDR, or XDR) and *rpoB* mutation at locus 531 were statistically associated with mutations in the *rpoC* gene (Table 2).

The sequencing of the RRDR of the *rpoB* gene of 151 RMP-resistant strains revealed that 144 (95.4%) of the isolates carried nonsynonymous mutations, 71 (47.0%) harbored point mutations at locus 531, 69 (45.7%) showed mutation S531L, and 2 (1.3%) had mutation S531W. Among the 71 strains with mutations at locus 531 of the *rpoB* gene, 36 (50.7%) also harbored *rpoC* mutation, which was significantly higher than that of isolates with no mutation or mutations at loci other than locus 531 of the *rpoB* gene ($P < 0.0001$, Table 2).

Factors associated with MIRU-VNTR clusters

According to results of MIRU-VNTR genotyping in this study, 45 (18.4%) isolates were distributed across 20 clusters, 17 of which were composed of 2 strains each, 1 cluster was formed by 3 strains, and 2 clusters were composed of 4 strains each.

To determine possible factors associated with MTB transmissibility, we compared the proportion of relative factors between isolates belonging to MIRU-VNTR clusters and isolates with a unique profile. Host factors, such as age, gender, and treatment history, and bacterial factors, including strain family, RMP phenotypic drug resistance profile, and *rpoB* and *rpoC* mutations, were included in the logistic regression model. Multivariate logistic regression analysis revealed that host factors, including age, gender, and treatment history, were not associated with MIRU-VNTR clusters (Table 3). In the MIRU-VNTR clusters, the proportion of Beijing family strains was higher than that of non-Beijing family ones. However, this difference was not statistically significant in the multivariate regression model ($P = 0.1238$). Compared with RMP-susceptible isolates, the proportions of isolates in the clusters increased with high levels of drug resistance but were not statistically significant ($P = 0.4091$, $P = 0.1613$). Similarly, we did not find any association between

Table 1 Profile of nonsynonymous mutations in the *rpoC* and *rpoB* genes of rifampin-resistant strains

Nonsynonymous (s) in the *rpoC* gene	Mutation (s) at codon (s)	No. (%) of strains	References	Mutations in RRDR of *rpoB* gene
E275G	275	1 (0.7)	Not reported	H526L
G332R	332	1 (0.7)	[14, 16, 21, 22]	S531L
G332S	332	1 (0.7)	[23, 16]	S531L
G388A	388	1 (0.7)	[15]	H526D
G433C	433	1 (0.7)	[15, 22]	S531L
M447V	447	1 (0.7)	Not reported	H526L
V483A	483	1 (0.7)	[14–16, 22–24]	S531L
V483G	483	16 (10.6)	[14–16, 21–24]	D516V (1 strain) S531W (1 strain) No mutation (1 strain) S531L (13 strains)
W484G	484	3 (2.0)	[15, 16, 19, 22]	S531L
D485Y	485	1 (0.7)	[14, 16, 22]	L511P
I491T	491	1 (0.7)	[14, 15, 19, 21, 22]	S531L
I491V	491	2 (1.3)	[15, 16, 22, 23]	S531L
A492V	492	1 (0.7)	[15]	S531L
L516P	516	2 (1.3)	[16, 19, 22, 23]	S531L
A521D	521	1 (0.7)	[15, 16, 22, 23]	S531L
A521V	521	1 (0.7)	Not reported	S531L
S561P	561	1 (0.7)	[15, 22, 24]	H526R
L566R	566	2 (1.3)	Not reported	S522L
N698K	698	3 (2.0)	[15, 16, 19, 22]	S531L
A701T	701	1 (0.7)	Not reported	TTC insert between loci 514 and 515
A788E	788	2 (1.3)	Not reported	H526y
T845A	845	1 (0.7)	Not reported	H526R
Y921C	921	1 (0.7)	Not reported	H526Y
D943G	943	1 (0.7)	[22]	H526N
Y979C	979	1 (0.7)	Not reported	H526D
I991S	991	1 (0.7)	Not reported	D516Y
V1039A	1039	1 (0.7)	Not reported	S531L
S1115L	1115	1 (0.7)	Not reported	S531L
V1252L	1252	1 (0.7)	[16, 22]	L533P, F505L
Q1160P, T812I	1160 and 812	1 (0.7)	Not reported	S531L, L511P
A519D, I997V	519 and 997	1 (0.7)	Not reported	S531L
E154D, A492P	154 and 492	1 (0.7)	Not reported	S531L

rpoB mutation and MIRU-VNTR clusters ($P = 0.8095$). We investigated the link between *rpoC* mutations and MTB transmissibility by comparing the proportion of isolates with and without *rpoC* mutations in the clusters. Approximately 25.4% of the isolates harbored *rpoC* mutation, which was slightly higher than that of isolates without *rpoC* mutation (16.1%). However, this value was not statistically significant ($P = 0.4519$, Table 3).

We used RMP-resistant isolates as a subset to assess if *rpoC* mutations facilitate the transmission of RMP-resistant strains and found that the proportions of isolates with and without *rpoC* mutation in the MIRU-VNTR clusters were 27.3% and 18.8%, respectively. This difference was not statistically significant (OR, 1.625, 95% CI, 0.742–3.560, $P = 0.2248$).

Discussion

This study examined the profile of compensatory mutations in the *rpoC* gene of MTB isolates from patients with pulmonary TB in two municipalities and four provinces of China and investigated the possible factors associated with the transmission of MTB. The results showed that 36.4% of the RMP-resistant MTB isolates harbored nonsynonymous *rpoC* mutations. Of the RMP-resistant isolates with

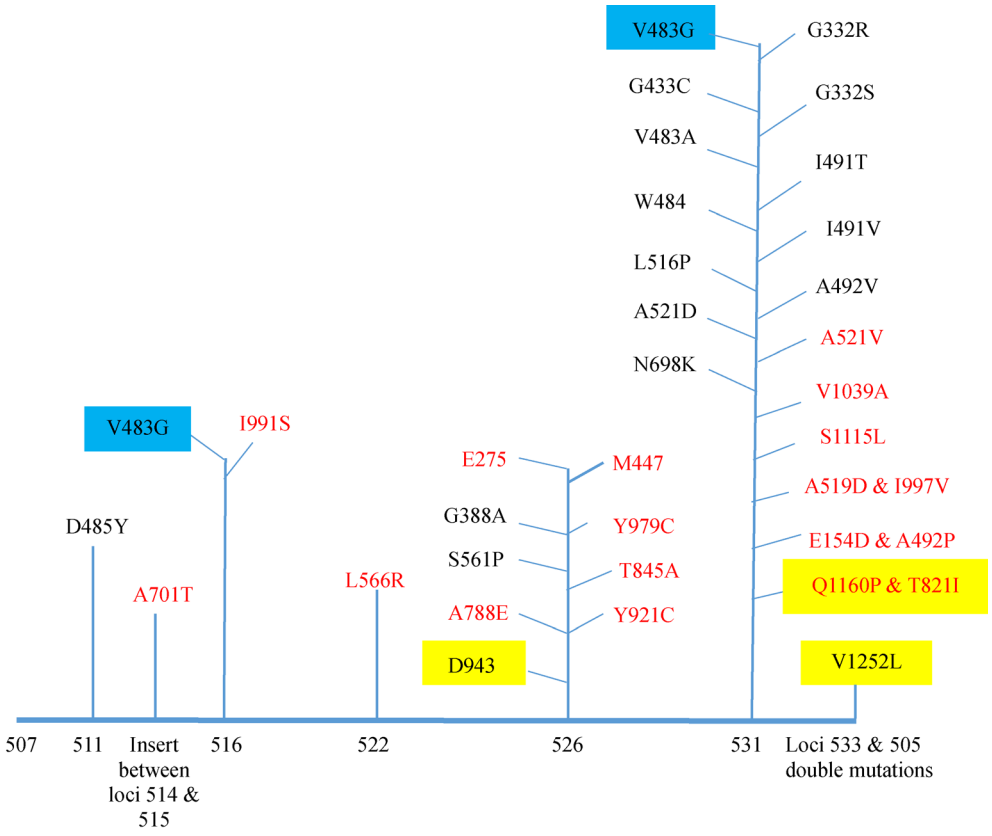

Fig. 1 Schematic of *rpoC* mutations at different loci of the RRDR of the *rpoB* gene. *rpoC* mutations in red indicate that the mutations have not been reported previously, and those in black indicate that the mutations have been reported. Mutations with a blue and yellow background indicate identical mutations and double mutations at the RRDR loci of the *rpoB* gene, respectively.

nonsynonymous mutations in the *rpoC* gene, 98.2% (54/55) harbored mutations in the RRDR of the *rpoB* gene. A recent study on MTB revealed that more than 30% of MDR clinical strains isolated from high-burden countries have compensatory mutations [19], and the proportion of compensatory mutations found in the present study is slightly larger than that reported in high-burden countries. Previous studies have revealed that RMP-resistant strains harbor *rpoC* mutations, but these mutations do not appear in any RMP-sensitive isolates [14,19–21]. In the current study, four nonsynonymous and three synonymous mutations were identified in RMP-sensitive isolates. These results are in accordance with those of a previous study conducted in China [15]. Such mutations should be studied in the future.

In this study, we identified 29 nonsynonymous single mutations and 3 double mutations in the *rpoC* gene. Although most of the mutations in *rpoC* have been previously reported [14–16,19,21–24], we identified 12 new point mutations and 3 double mutations in *rpoC*.

We analyzed the diversities of *rpoC* mutations in different codons of the *rpoB* gene and found that different loci (511, 516, 522, 526, and 531) of the *rpoB* gene exhibited varying patterns of compensatory mutations of *rpoC*, except for V483G of *rpoC* mutation, which was identified at loci 516 and 531 of the *rpoB* gene. We identified 9 and 18 different types of *rpoC* mutations at locus 526 (including double mutation H526N and M515V) and locus 531 (including double mutation S531L and L511P) of *rpoB*, respectively. Mutation with S531L had a different type of *rpoC* mutation and a high level of diversity.

Multivariate logistic regression analysis revealed a statistically significant association between *rpoC* mutation and RMP resistance and mutations at locus 531 of the *rpoB* gene. This result is in accordance with those of previous studies [14,15,22]. In the current study, strains carrying mutations at locus 531 of *rpoB* were frequently found in the isolated strains. Approximately 50.0% of isolates with point mutations at locus 531 of the *rpoB* gene harbored *rpoC* mutation, which was significantly higher than that of no mutation or mutation(s) at loci other than locus 531. An explanation for this result is compensatory mutation [19,25,26]. Previous studies have strongly implied that

Table 2 Factors associated with nonsynonymous mutations in the *rpoC* gene

Factors	No. (%) of isolates		Univariate analysis		Multivariate analysis	
	No *rpoC* mutation	*rpoC* with mutation	OR (95%CI)	*P* value for OR	Adjusted OR (95% CI)	*P* value for adjusted OR
Age						
<30 years	45 (77.59)	13 (22.41)	1.0		1.0	
30–60 years	102 (73.91)	36 (26.0)	1.222 (0.592–2.522)	0.5881	1.711 (0.720–4.065)	0.2238
⩾60 years	38 (79.17)	10 (20.83)	0.911 (0.359–2.310)	0.8443	1.283 (0.432–3.812)	0.6537
Gender						
Male	137 (74.86)	46 (25.14)	1.0		1.0	
Female	48 (78.69)	13 (21.31)	0.807 (0.401–1.621)	0.5462	0.835 (0.375–1.862)	0.6600
Treatment history						
New	94 (75.20)	31 (24.80)	1.0		1.0	
Retreatment	90 (76.27)	28 (23.73)	0.943 (0.524–1.697)	0.8457	0.715 (0.357–1.430)	0.3428
Strain family						
Non-Beijing	37 (78.72)	10 (21.28)	1.0		1.0	
Beijing	149 (75.25)	49 (24.75)	1.217 (0.564–2.627)	0.6173	0.864 (0.342–2.186)	0.7581
RMP susceptibility						
Susceptible	90 (95.74)	4 (4.26)	1.0		1.0	
Monoresistant or MDR	64 (62.14)	39 (37.86)	13.706 (4.666–40.263)	<0.0001	8.415 (2.658–26.642)	0.0003
Pre-XDR or XDR	32 (66.67)	16 (33.33)	11.246 (3.499–36.141)	<0.0001	6.968 (1.901–25.540)	0.0034
rpoB mutation						
No mutation or mutation(s) at loci other than locus 531	150 (86.71)	23 (13.29)	1.0		1.0	
Point mutation at locus 531 only	36 (50.00)	36 (50.00)	6.522 (3.448–12.334)	<0.0001	3.464 (1.700–7.058)	0.0006

RMP, rifampin; MDR, multidrug resistant; XDR, extensively drug-resistant tuberculosis.

rpoC and *rpoA* mutations in RMP-resistant MTB are fitness compensatory mutations, and strains with these compensatory mutations show high competitive fitness not only *in vitro* but also *in vivo* [27,28]. Another possible reason may be related to the structure of DNA-dependent RNA polymerase. In bacteria, this enzyme comprises multiple subunits (α2ββ′ω), among which the two largest subunits (β′ and β) that contain collinearly arranged segments of a conserved sequence are encoded by *rpoC* and *rpoB*. The evolutionary relationship between β′ and β subunits may be particularly strong. Polymorphisms in the subunits of an enzyme may interact closely with one other to reduce initial fitness defects caused by drug-resistant conferring mutation [19,29,30].

The influence of bacterial factors, such as genetic, clinical, and demographic characteristics of patients, on MTB clustering was analyzed in this study. In contrast with a previous study, we did not find patients infected with the Beijing family strain to be clustered [31]. Our study did not identify any risk factors, such as RMP resistance profile, *rpoB* mutation, or treatment history of patients that were statistically significant associated with MIRU-VNTR clusters. *rpoC* mutation was not a risk factor significantly associated with MIRU-VNTR clusters, a result that is consistent with that of a recent report [16,23] but contrary to those in other studies [14,15]. These heterogeneous results may be due to differences in study setting, study object, methodologies applied, and quality of the TB control program.

Our study has several limitations. First, the 15 loci for the MIRU-VNTR analysis of MTB may have decreased the discrimination power and thus overestimated the cluster rate in this study. Second, according to previous studies, *rpoC* mutations are more frequent than *rpoA* gene mutations [14].Thus, *rpoC* was sequenced for mutation detection, and *rpoA* gene mutation was not. Third, according to the findings of a previous study [32], 95% of RMP-resistant mutations are present in the *rpoB* gene, and the majority of the mutations in the *rpoB* gene are within RRDR. Thus, this study focused on sequencing the RRDR of the *rpoB* gene and may have missed mutations located outside of the RRDR of the *rpoB* gene.

In summary, this study found a high proportion of *rpoC* mutations in RMP-resistant MTB isolates prevalent in China. The results showed a significant association between *rpoC* and *rpoB* mutations at locus 531, suggesting

Table 3 Logistic regression analysis for risk factors associated with MIRU-VNTR clusters

Factors	No. (%) of isolates		Univariate analysis		Multivariate analysis	
	Not in the VNTR cluster	In the VNTR cluster	OR (95% CI)	*P* value for OR	Adjusted OR (95% CI)	*P* value for adjusted OR
Age						
<30 years	45 (77.59)	13 (22.41)	1.0		1.0	
30–60 years	102 (73.91)	36 (26.09)	1.088 (0.500–2.364)	0.8322	1.528 (0.657–3.554)	0.3245
⩾60years	38 (79.17)	10 (20.83)	0.610 (0.208–1.794)	0.3695	0.787 (0.254–2.442)	0.6789
Gender						
Male	151 (82.51)	32 (17.49)	1.0		1.0	
Female	48 (78.69)	13 (21.31)	1.278 (0.621–2.630)	0.5053	1.232 (0.576–2.636)	0.5905
Treatment history						
New	97 (77.60)	28 (22.40)	1.0		1.0	
Retreatment	101 (85.59)	17 (14.41)	0.583 (0.300–1.133)	0.1113	0.500 (0.245–1.023)	0.0578
Strain family						
Non-Beijing	43 (91.49)	4 (8.51)	1.0		1.0	
Beijing	157 (79.29)	41 (20.71)	2.807 (0.953–8.270)	0.0612	2.401 (0.787–7.323)	0.1238
RMP phenotype						
RMP sensitive	82 (87.23)	12 (12.77)	1.0		1.0	
Monoresistant or MDR	82 (79.61)	21 (20.39)	1.750 (0.808–3.789)	0.1556	1.457 (0.596–3.562)	0.4091
Pre-XDR or XDR	36 (75.00)	12 (25.00)	2.278 (0.934–5.552)	0.0702	2.133 (0.739–6.156)	0.1613
rpoB mutation						
No mutation or mutation(s) at locus other than locus 531	145 (83.82)	28 (16.18)	1.0		1.0	
Point mutation at locus 531 only	55 (76.39)	17 (23.61)	1.601 (0.813–3.153)	0.1735	1.109 (0.479–2.567)	0.8095
rpoC mutation						
No *rpoC* mutation	156 (83.87)	30 (16.13)	1.0		1.0	
rpoC mutation	44 (74.58)	15 (25.42)	1.773 (0.877–3.585)	0.1111	1.362 (0.609–3.047)	0.4519

RMP, rifampin; MDR, multidrug resistant; XDR, extensively drug-resistant tuberculosis.

that *rpoC* mutation may reduce the fitness cost of RMP-resistant MTB, leading to compensated transmissibility. However, the finding on the lack of significant association of *rpoC* mutation with the clustering of MTB isolates does not support the role of *rpoC* mutation in transmissibility.

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Compliance with ethics guidelines

Shengfen Wang, Yang Zhou, Bing Zhao, Xichao Ou, Hui Xia, Yang Zheng, Yuanyuan Song, Qian Cheng, Xinyang Wang, and Yanlin Zhao declare that they have no conflict of interest. This study was approved by the ethical review board of the Chinese Center for Disease Control and Prevention, and informed consent was obtained from all individual participants involved in this study.

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