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Drug-resistance characteristics, genetic diversity, and transmission dynamics of multidrug-resistant or rifampicin-resistant *Mycobacterium tuberculosis* from 2019 to 2021 in Sichuan, China

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Abstract

Background Multidrug- or rifampicin-resistant tuberculosis (TB; MDR/RR-TB) is a significant public health threat. However, the mechanisms involved in its transmission in Sichuan, China are unclear. To provide a scientific basis for MDR/RR-TB control and prevention, we investigated the drug-resistance characteristics, genetic diversity, and transmission dynamics and analyzed the demographic and clinical characteristics of patients to identify risk factors for the acquisition of MDR/RR-TB in Sichuan, Western China.

Methods Whole-genome sequencing was performed using a sample comprised of all MDR/RR-TB strains isolated from patients with pulmonary TB (≥ 15 years) at the 22 surveillance sites in Sichuan province between January 2019 and December 2021, to analyze genotypic drug resistance and genetic diversity. Moreover, we performed statistical analyses of the epidemiological characteristics and risk factors associated with the transmission dynamics of MDR/RR-TB.

Results The final analysis included 278 MDR/RR TB strains. Lineage 2.2, the major sub-lineage, accounted for 82.01% (228/278) of isolates, followed by lineage 4.5 (9.72%, 27/278), lineage 4.4 (6.83%, 19/278), and lineage 4.2 (1.44%, 4/278). The drug resistance rates, ranging from high to low, were as follows: isoniazid (229 [82.37%]), streptomycin (177 [63.67%]), ethambutol (144 [51.80%]), pyrazinamide (PZA, 119 [42.81%]), fluoroquinolones (FQs, 93 [33.45%]). Further, the clofazimine, bedaquiline, and delamanid resistance rates were 2.88, 2.88, and 1.04%, respectively. The gene composition cluster rate was 32.37% (90/278). In addition, 83.81% (233/278) of MDR/RR-TB cases were determined to be likely caused by transmission. Finally, patients infected with lineage two strains and strains with the KatG S315T amino acid substitution presented a higher risk of MDR/RR-TB transmission.

Conclusion Transmission plays a significant role in the MDR/RR-TB burden in Sichuan province, and lineage 2 strains and strains harboring KatG S315T have a high probability of transmission. Further, high levels of FQ and PZA drug resistance suggest an urgent need for drug susceptibility testing prior to designing therapeutic regimens. New anti-TB drugs need to be used standardly and TB strains should be regularly monitored for resistance to these drugs.

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Keywords Multidrug-resistant or rifampicin-resistant tuberculosis (MDR/RR-TB), Whole-genome sequencing (WGS), Genetic diversity, Transmission dynamics

Background

Tuberculosis (TB) was the second leading cause of death from a single infectious agent worldwide in 2022, following corona-virus disease 2019 (COVID-19) [1]. Moreover, drug-resistant tuberculosis (DR-TB), particularly multidrug-resistant or rifampicin-resistant TB (MDR/RR-TB), remains a significant public health threat, and this requires treatment with second-line medications for up to 2 years, with a treatment success rate of only 63% [1]. China has a high burden of TB and MDR/RR-TB, with 748,000 new TB cases, approximately 30,000 of which were MDR/RR-TB, in 2022 [1].

Sichuan province, located in western China, has the highest prevalence of TB and is lagging behind the eastern coastal provinces of China in reducing its TB burden [2]. The DR-TB situation in Sichuan is also serious. Further, the MDR-TB rates among new and retreated patients (5.45 and 23.02%, respectively) [3] were similar to the baselines reported in a national survey in 2007 (5.7 and 25.6%, respectively) [4] and higher than the global averages from 2010 to 2020 (3–4% and 18–21%, respectively) [5]. Therefore, understanding the factors that influence the prevalence of DR-TB in Sichuan and formulating precise strategies to mitigate this epidemic is imperative.

Previous studies have shown that the transmission of MDR-TB strains plays a significant role in the burden of MDR-TB in China [6]. Therefore, it is of great significance to understand the transmission and the risk factors of MDR-TB acquisition in the region for the formulation of local anti-TB policies. Whole-genome sequencing (WGS) is an effective tool that is widely used to predict drug resistance, perform phylogenetic classification, investigate transmission chains, identify mixed infections, and reveal the evolution of the *Mycobacterium tuberculosis* complex [7, 8]. Several studies have used WGS to investigate the local transmission of TB or MDR/RR-TB and the influencing factors, focusing on central and eastern areas of China, such as Shanghai, Shenzhen, Hubei, and Hunan [6, 9–11]. However, the mechanisms involved in MDR/RR-TB transmission in Sichuan have not yet been investigated. To provide a scientific basis for DR-TB control and prevention, we conducted a retrospective study of MDR/RR-TB in Sichuan Province from 2019 to 2021 using WGS to better understand the drug-resistance characteristics, genetic diversity, transmission dynamics, and risk factors.

Methods

Sample collection

This was a retrospective study based on routine drug resistance surveillance in Sichuan province. The drug resistance monitoring program set up 22 drug resistance monitoring sites, with one county selected as a surveillance site in each city, except for Mianyang City, where two sites were set up. The study sample comprised all MDR/RR-TB strains isolated from patients with pulmonary TB (≥ 15 years), with sputum smear-positive and/or molecular biology-positive samples, who visited local designated hospitals or dispensaries at the 22 surveillance sites in Sichuan province between January 2019 and December 2021. Patients with pulmonary tuberculosis (PTB) were diagnosed following the "Criteria for PTB Diagnosis" of China (WS288-2017) [12]. Sputum samples were collected from all patients with PTB (≥ 15 years) with sputum smear-positive and/or molecular biology-positive samples.

Sample culture

Each sample was liquefied using 4% NaOH and inoculated into tubes containing acidified Löwenstein–Jensen medium for further culture, and the identification of *M. tuberculosis* included *p*-nitrobenzoic acid testing. All MDR/RR-TB strains were previously identified using the proportion method on Lowenstein–Jensen medium containing rifampicin at 40 mg/mL and then stored at − 80 °C. The proportion method is most commonly used for testing the susceptibility of *M. tuberculosis* complex isolates. In this method, the ratio of the number of colonies on the medium containing the anti-TB agent to that on the medium without the anti-TB agent is then calculated, and the proportion is expressed as a percentage. For most anti-TB agents, a 1% critical proportion differentiating the proportion of resistant organisms within a particular strain is used to determine clinically significant resistance to a particular drug. Subsequently, MDR/RR-TB strains were tested using WGS.

Patient information

Patient information was obtained from the Tuberculosis Information Management System of China, and it included demographic information (sex, age, occupation, and census registry) and clinical characteristics (history of previous TB treatment, concomitant disease, visiting hospital delay, health system delay, and patient source).

WGS

Genomic DNA was extracted and purified using the cetyltrimethylammonium bromide method. Libraries were constructed on an Illumina platform using the FS DNA Lib Prep Kit V6 (RK20259). The samples were then sequenced using an Illumina NovaSeq 6000 sequencer. Referring to a previous study [13], the whole genome was analyzed to identify TB strain genotypes. The drug-resistance spectrum of each strain based on 16 types of anti-TB drugs was predicted according to the latest mutation catalog recommended by the World Health Organization [14]. A phylogenetic tree was constructed using the maximum likelihood method and visualized using iTOL (https://itol.embl.de/). The genetic distance was calculated to analyze TB transmission characteristics in the study areas, and genomic transmission clusters were defined using 12 SNPs as cutoff values [6]. The gene composition clustering rate was defined as the ratio of the number of clustered strains to the total number of strains.

Statistical analysis

SPSS version 27 software (SPSS Inc., Chicago, Illinois) was used for the statistical analysis. The chi-square test or Fisher's exact test was used to compare differences in the categorized variables between the groups. Univariable and multivariable logistic regression analyses were performed to identify the risks of relevant factors for the transmission clusters of MDR/RR-TB strains, and the odds ratio (OR) and 95% confidence intervals (95% CIs) of each risk factor were calculated. Results with a p-value less than 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics

In total, 4866 positive cultures were collected between January 2019 and December 2021 at 22 drug resistance monitoring sites. Of the patients from which these samples were derived, 330 (6.78%) were classified as having MDR/RR-TB using the proportion method, and these samples were subjected to WGS. After excluding 31 duplicate strains from the same patient and 21 strains that could not be sequenced, 278 representative strains were included in the final analysis (Fig. 1). Most patients with MDR/RR-TB (76.98%, 214/278) were male. The median age of the patients was 52 years (range, 16–58 years), and most patients (67.63%, 188/278) were older than 45 years. The percentage of patients of Han nationality was 97.48% (271/278), and 78.55% (216/275) were farmers.

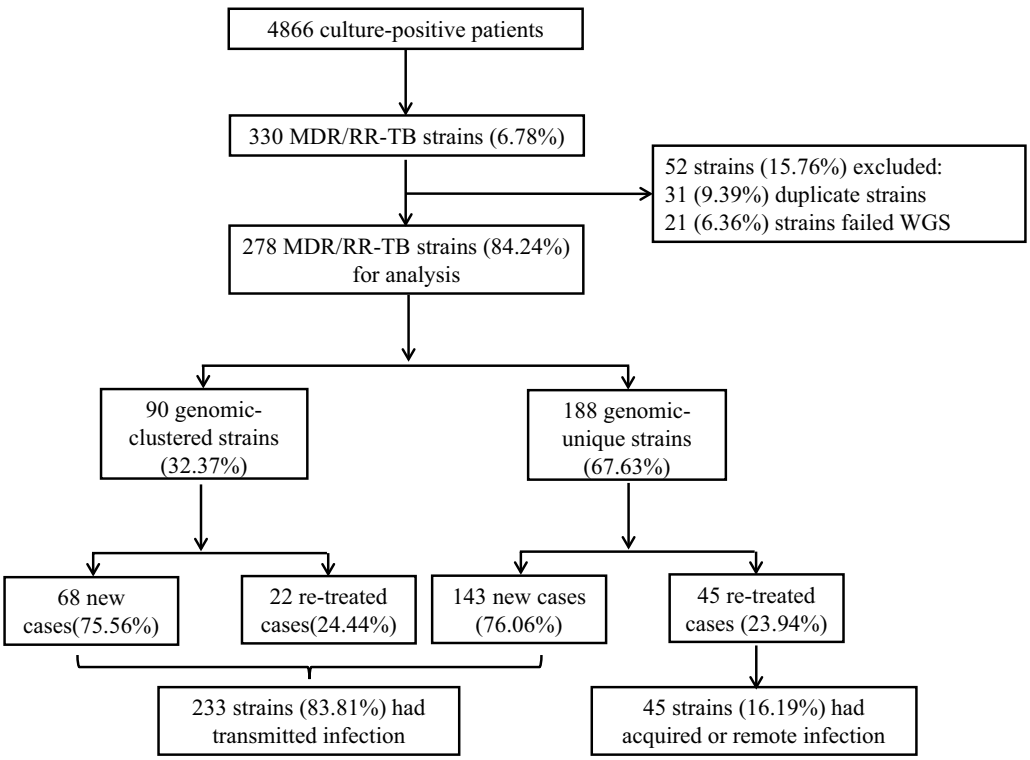

Fig. 1 Classification of MDR/RR-TB based on treatment history and genomic analysis. MDR/RR-TB, multidrug-resistant or rifampicin-resistant tuberculosis; WGS, whole-genome sequencing

Moreover, most patients were native residents (88.36%, 243/275), and 231 patients (84.00%, 231/275) had no concomitant disease. Most patients (75.90%, 211/278) were newly diagnosed, whereas 67 (24.10%) had previously received treatment. Information on the HIV status of the patients was not collected. The median delay in hospital visits was 18 days, the median health system delay was 1 day, and most patients were identified by referral. The detailed demographic information and clinical characteristics of the study population are presented in Table 1.

Genetic structure and drug resistance

A phylogenetic tree was constructed based on the whole-genome sequences of 278 MDR/RR-TB strains from Sichuan Province (Fig. 2). Two main lineages were identified; specifically, 82.01% (228/278) of the strains were assigned to lineage 2 (East Asian genotype), and 17.99% (50/278) were assigned to lineage 4 (Euro-American genotype). Regarding sublineages, the majority (82.01%, 228/278) belonged to lineage 2.2. The remaining 27 strains belonged to lineage 4.5 (9.72%, 27/278); 23 strains belonged to lineage 4.4 (6.83%, 19/278), and four strains belonged to lineage 4.2 (1.44%,4/278).

Table 1 Demographic information and clinical characteristics of 278 patients with MDR/RR-TB in Sichuan, China

Characteristic	Total cases (N = 278)		New cases (N = 211)		Re-treated cases (N = 67)	
	n	%	n	%	n	%
Sex						
Male	214	76.98	157	74.41	57	85.07
Female	64	23.02	54	25.59	10	14.93
Age group						
≤ 24	24	8.63	19	9.00	5	7.46
25–44	66	23.74	51	24.17	15	22.39
45–65	128	46.04	90	42.65	38	56.72
> 65	60	21.58	51	24.17	9	13.43
Nationality						
Han nationality	271	97.48	206	97.63	65	97.01
Other	7	2.52	5	2.37	2	2.99
Occupation*						
Farmer	216	78.55	159	75.71	57	87.69
Students and faculty	15	5.45	14	6.67	1	1.54
Other	44	16.00	37	17.62	7	10.77
*Census registry**						
Resident	243	88.36	184	87.62	59	90.77
Migrant	32	11.64	26	12.38	6	9.23
*Concomitant disease**						
Yes	44	16.00	33	15.71	11	16.92
No	231	84.00	177	84.29	54	83.08
*Visiting hospital delay**						
< 14 days	125	45.45	97	46.19	28	43.08
≥ 14 days	150	54.55	113	53.81	37	56.92
*Health system delay**						
< 14 days	241	87.64	187	89.05	54	83.08
≥ 14 days	34	12.36	23	10.95	11	16.92
*Patient source**						
Clinic visit due to symptoms	80	29.09	56	26.67	24	36.92
Tracing	63	22.91	55	26.19	8	12.31
Referral	121	44.00	91	43.33	30	46.15
Other	11	4.00	8	3.81	3	4.62

Three cases had missing information regarding the census registry, concomitant disease, health system delay, visiting hospital delay, and patient source (*). MTB/RR-TB, multidrug-resistant or rifampicin-resistant tuberculosis

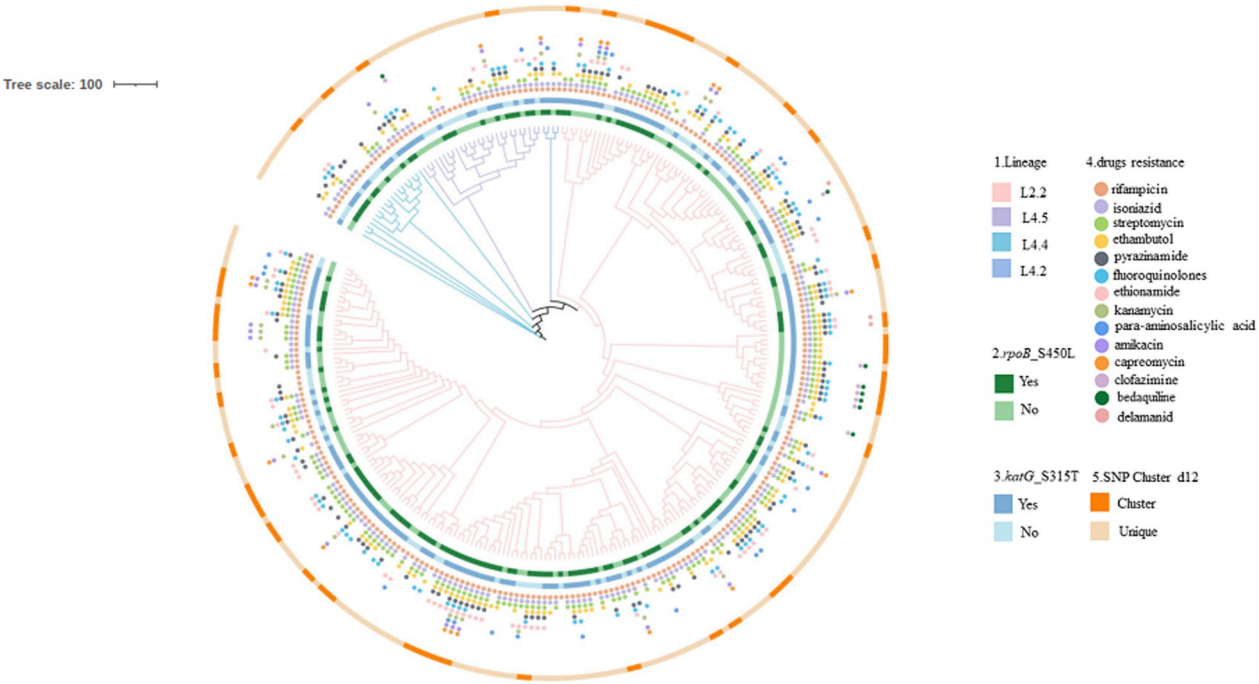

Fig. 2 Phylogenetic tree of 278 MDR/RR-TB strains isolated in Sichuan. *Note* the different colors on the branches indicate different sub-lineages. The first outer circle indicates the presence or absence of RpoB S450L. The second outer circle indicates the presence or absence of KatG S315T. The small circles of different colors on the outer middle ring indicate drug resistance. The outer-most circle indicates genomic-clustered strains differing by ≤ 12 SNPs. L2.2, lineage 2.2; L4.5, lineage 4.5; L4.4, lineage 4.4; L4.2, lineage 4.2. MDR/RR-TB, Multidrug-resistant or rifampicin-resistant tuberculosis

Resistance to 16 anti-TB drugs was detected, and the rates of resistance to different drugs, from high to low, were as follows: isoniazid (INH, 229 [82.37%]), streptomycin (SM, 177 [63.67%]), ethambutol (EMB, 144 [51.80%]), pyrazinamide (PZA, 119 [42.81%]), fluoroquinolones (FQs, 93 [33.45%]), ethionamide (ETO, 71 [25.54%]), kanamycin (KM, 24 [8.63%]), para-aminosalicylic acid (PAS, 23[8.27%]), amikacin (AM, 22 [7.91%]), capreomycin (CM, 18 [6.47%]), clofazimine (CFZ,8 [2.88%]), bedaquiline (BDQ, 8 [2.88%]), and delamanid (DEL, 3 [1.08%]). Strains resistant to cycloserine and linezolid (LZD) were also identified. The rates of MDR-TB, pre-extensively drug-resistant (pre-XDR-TB), and extensively drug-resistant (XDR-TB) strains were 82.37% (229/278), 33.45% (144/278), and 2.15% (6/278), respectively. Moreover, all 278 phenotypic MDR/RR-TB strains had detectable mutations in the *rpoB* gene. The most common drug resistance-associated mutation in RIF-resistant strains was RpoB S450L (136/278, 48.92%), and 188 INH-resistant strains had the drug resistance-associated mutation KatG S315T. Among the 278 MDR/RR-TB strains, resistance to the different drugs is shown in Fig. 3.

Genetic distance and clustering analysis

Overall, 90 MDR/RR-TB strains were divided into 39 clusters, ranging in size from two to seven strains, suggesting recent transmission. The gene composition cluster rate was 32.37% (90/278). Moreover, most clusters contained two strains, accounting for 71.11% of the clustered cases (64/90) (Table 2). A previous study showed that the presence of TB among new cases suggests the transmission of TB strains [6]. Therefore, cases of newly diagnosed MDR/RR-TB were combined with those in the genomic clusters; 83.81% (233/278) of the cases were likely caused by transmission in our study (Fig. 1).

The results of the maximum-likelihood tree of clustered strains showed 18 clusters of strains spanning years, and only the largest cluster (C14) included strains spanning more than 3 years (Fig. 4). In total, 33.33% of the clusters (13/39) included strains with different drug-resistance spectra. Drug-resistance profiles progressively increased in the strains of four clusters (C7, C12, C16, and C32) in relation to chronology, including the C7 cluster, with increased drug-resistance profiles against FQs, the C12 cluster, against EMB, PZA, and FQs, C16, against PZA and FQs, and C32, against EMB. The C37 cluster

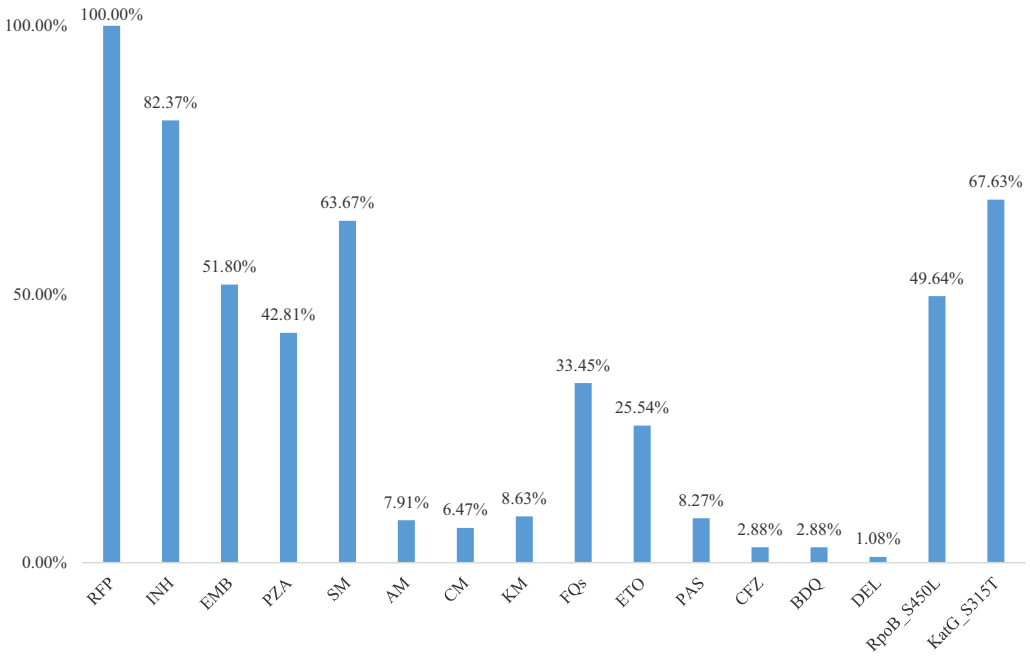

Fig. 3 Drug resistance and gene mutation types in 278 MDR/RR-TB strains. *Note* The X-axis was each drug based on drug mutations and gene mutation types. The Y-axis was the percentage of isolates containing the drug mutation. MDR/RR-TB, multidrug- or rifampicin-resistant tuberculosis; *RFP*, rifampicin; *INH*, isoniazid; *EMB*, ethambutol; *PZA*, pyrazinamide; *SM*, streptomycin; *AM*, amikacin; *CM*, capreomycin; *KM*, kanamycin; *FQs*, fluoroquinolones; *ETO*, ethionamide; *PAS*, para-aminosalicylic acid; *CFZ*, clofazimine; *BDQ*, bedaquiline; *DEL*, delamanid

Table 2 Cluster size and the number of genomic clusters of 90 MDR/RR-TB strains

No of strains in clusters (N)	No of clusters (N)	No of strains (N)	Proportion (%)
2	32	64	71.11
3	5	15	16.67
4	1	4	4.44
7	1	7	7.78
Total	39	90	100.00

MDR/RR-TB, multidrug-resistant or rifampicin-resistant tuberculosis

indicated that the descendants had increased drug-resistance profiles against EMB and decreased drug-resistance profiles against PAS. The strains from six clusters (C1, C4, C6, C10, C15, and C21) collected in the same year exhibited different drug-resistance profiles. Further, the ancestral strains of two clusters (C3 and C14) showed broader drug-resistance profiles than their descendants.

Risk factors for clusters

To analyze the characteristics of the MDR/RR-TB strains that were more prone to transmission, we compared demographic and clinical characteristics based on the clustered and non-clustered strains (Table 3). The clustering rate in strains belonging to lineage 2 was significantly higher than that of other MDR-TB lineages (35.96% vs. 16.00%). However, there was no significant difference between the clustering rates of drug-resistance profiles among TB strains, except for INH-, SM-, AM-, and PZA-resistant strains. We also analyzed the influence of the most common drug-resistance-associated mutations in RIF-resistant- and INH-resistant strains based on clustering. The strains with KatG S315T had a higher probability of genetic clustering, whereas the RpoB S450L mutation was not associated with strain clustering. The clustering rate based on students and faculty members was higher than that based on farmers and other occupations (46.67% vs. 31.54%, respectively); however, the difference was not statistically significant. No other patient characteristics were associated with clustering.

Factors significantly associated with clustering were included in the univariable and multivariable logistic regression models, as shown in Fig. 5. Based on the univariable logistic analysis, the risk factors independently related to the recent transmission of MDR/RR-TB included patients with lineage 2 strains (OR 2.949; 95% CI 1.321–6.582), resistance to PZA (OR 1.883; 95% CI 1.133–3.131), resistance to SM (OR 1.764; 95% CI 1.022–3.044), resistance to AM (OR 2.738; 95% CI 1.135–6.605), and KatG S315T (OR 2.483; 95% CI 1.370–4.498), whereas INH-resistant strains had no significant transmission advantage. These factors were included in the

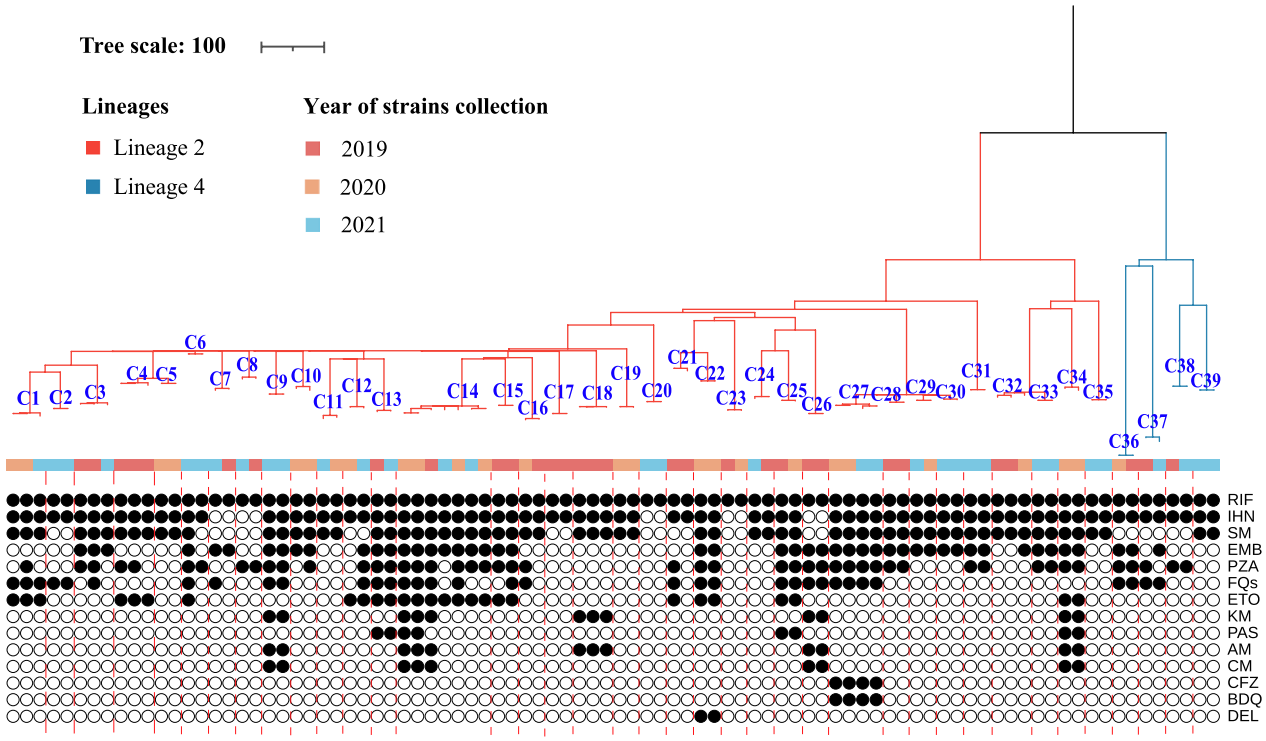

Fig. 4 Maximum-likelihood tree of 90 MDR/RR-TB strains within 39 clusters and their drug-resistance profiles. *Note* the red dotted lines indicate boundaries of individual clusters; cluster 1–39 was labeled as C1-C39; circles filled with black indicate drug-resistant strains, whereas empty circles indicate drug-susceptible strains; MDR/RR-TB, multidrug-resistant or rifampicin-resistant tuberculosis; *RFP*, rifampicin; *INH*, isoniazid; *SM*, streptomycin; *EMB*, ethambutol; *PZA*, pyrazinamide; *FQs*, fluoroquinolones; *ETO*, ethionamide; *KM*, kanamycin; *PAS*, para-aminosalicylic acid; *AM*, amikacin; *CM*, capreomycin; *CFZ*, clofazimine; *BDQ*, bedaquiline; *DEL*, delamanid

multivariable logistic regression model. The transmission risk in patients infected with lineage 2 strains was 3.044 (1.253–7.394)-fold higher than that in patients infected with lineage 4 strains. Moreover, MDR/RR-TB strains with KatG S315T had a significantly increased probability of clustering (OR 2.360, 95% CI 1.094–5.088).

Spatial distribution of clustered patient samples

We collected the addresses of clustered samples from patients to describe their geographic distribution. Patient addresses for 38 of 39 clusters were collected. Five clusters (12.82%) were from the same town, 21 (53.85%) were from different towns of the same county, and 12 (30.77%) were from different counties.

Discussion

To the best of our knowledge, this is the first molecular epidemiological study using WGS technology to characterize MDR/RR-TB drug resistance, genetic diversity, and transmission during a 3-year interval in Sichuan, China. Based on the WGS analysis of 278 patient samples, the prevalent MDR/RR-TB strains belonged to lineage 2.2, accounting for 82.01% of samples, and the clustering rate was 32.37% in Sichuan. Moreover, 83.81% (233/278) of MDR/RR-TB cases were likely caused by transmission. Patients infected with lineage 2 strains and strains with KatG S315T also presented a higher risk of MDR/RR-TB transmission.

In our study, 82.37% of the RR-TB strains were MDR-TB, similar to that reported in Hunan (83%) [15]. In total, 33.45% of MDR/RR-TB cases were found to have additional resistance to FQs, known as pre-XDR-TB. FQs are the most valuable second-line anti-TB agents, particularly used for treating MDR-TB [16]. We found a substantial decrease in the rate of MDR-TB resistance to FQs (36.68%, 84/229) compared to that in our previous study (48.65%) in Sichuan from 2016 to 2017 [3], but this rate was even higher than the national average (30%) [17]. The data further showed a reduction in FQ resistance among MDR-TB strains isolated from patients in Sichuan, as a result of the standardization of antibiotic usage. PZA is an essential component of first-line TB drugs owing to its unique mechanism of action against MDR-TB, especially before a new MDR/RR-TB treatment regimen composed of BDQ and LZD can be implemented throughout China [18]. Moreover, drug susceptibility testing (DST)

Table 3 Demographic, bacteriological, and clinical characteristics of genomic-clustered and non-clustered tuberculosis in Sichuan, China

Characteristics	Total (N = 278)	Genomic-clustered		Genomic-unique		χ^2	*P*
		N	%	N	%		
Sex						0.152	0.697
Male	214	68	31.78	146	68.22		
Female	64	22	34.38	42	65.63		
Age group						0.318	0.957
≤ 24	24	9	37.50	15	62.50		
25–44	66	21	31.82	45	68.18		
45–65	128	41	32.03	87	67.97		
> 65	60	19	31.67	41	68.33		
Nationality						0.36	0.848
Han	271	87	32.10	184	67.90		
Other	7	3	42.86	4	57.14		
Occupation						1.483	0.350
Students and faculty	15	7	33.33	8	66.67		
Other	260	82	28.81	178	71.19		
Census registry						0.897	0.344
Resident	243	81	33.33	162	66.67		
Migrant	32	8	25.00	24	75.00		
Patient classification						0.009	0.926
New cases	211	68	32.23	143	67.77		
Re-treated cases	67	22	32.84	45	67.16		
Concomitant disease						0.19	0.663
Yes	44	13	29.55	31	70.45		
No	231	76	32.90	155	67.10		
Visiting hospital delay						0.434	0.51
< 14 days	125	43	34.40	82	65.60		
≥ 14 days	150	46	30.67	104	69.33		
Health system delay						1.383	0.24
< 14 days	241	81	33.61	160	66.39		
≥ 14 days	34	8	23.53	26	76.47		
Patient source						7.01	0.068
Clinic visit due to symptoms	80	31	38.75	49	61.25		
Tracing	63	25	39.68	38	60.32		
Referral	121	29	23.97	92	76.03		
Other	11	4	36.36	7	63.64		
Lineage						7.466	0.006
Lineage 2	228	82	35.96	146	64.04		
Lineage 4	50	8	16.00	42	84.00		
INH						3.89	0.049
R	229	80	34.93	149	65.07		
S	49	10	20.41	39	79.59		
EMB						0.126	0.723
R	144	48	33.33	96	66.67		
S	134	42	31.34	92	68.66		
PZA						6.025	0.014
R	119	48	40.34	71	59.66		
S	159	42	26.42	117	73.58		
SM						4.209	0.040

Table 3 (continued)

Characteristics	Total (N = 278)	Genomic-clustered		Genomic-unique		χ^2	*P*
		N	%	N	%		
R	177	65	36.72	112	63.28		
S	101	25	24.75	76	75.25		
CM						2.731	0.098
R	18	9	50.00	9	50.00		
S	260	81	31.15	179	68.85		
AM						5.364	0.021
R	22	12	54.55	10	45.45		
S	256	78	30.47	178	69.53		
KM						3.727	0.054
R	24	12	50.00	12	50.00		
S	254	78	30.71	176	69.29		
FQs						1.118	0.29
R	93	34	36.56	59	63.44		
S	185	56	30.27	129	69.73		
ETO						1.392	0.238
R	71	27	38.03	44	61.97		
S	207	63	30.43	144	69.57		
PAS						0.066	0.797
R	23	8	34.78	15	65.22		
S	255	82	32.16	173	67.84		
CFZ						0.278	0.237
R	8	4	50.00	4	50.00		
S	270	86	31.85	184	68.15		
BDQ						0.278	0.237
R	8	4	50.00	4	50.00		
S	270	86	31.85	184	68.15		
DEL						1.000	0.687
R	3	1	33.33	2	66.67		
S	275	88	32.00	187	68.00		
XDR-TB						0.089	0.089
Yes	6	4	66.67	2	33.33		
No	272	86	31.62	186	68.38		
KatG S315T						9.308	0.002
Yes	188	72	38.30	116	61.70		
No	90	18	20.00	72	80.00		
RpoB S450L						0.255	0.613
Yes	136	46	33.82	90	66.18		
No	142	44	30.99	98	69.01		

INH, isoniazid; *EMB*, ethambutol; *PZA*, pyrazinamide; *SM*, streptomycin; *CM*, capreomycin; *AM*, amikacin; *KM*, kanamycin; *FQs*, fluoroquinolones; *ETO*, ethionamide; *PAS*, para-aminosalicylic acid; *CFZ*, clofazimine; *BDQ*, bedaquiline; *DEL*, delamanid; *XDR-TB*, extensively drug-resistant tuberculosis

for PZA is not often performed in routine testing, and the prevalence of PZA resistance in Sichuan is unknown. Our data showed a PZA-resistance rate among MDR/RR-TB as high as 40.29%. Meanwhile, the results of a previous global meta-analysis showed a PZA-resistance rate among MDR-TB patients of 57% [19], and the rate in China was reported to be 43.5% [20]. Of note is that, we also observed resistance to CFZ, BDQ, and DEL. From 2019 to 2021, only one of the 21 designated MDR treatment hospitals in Sichuan used BDQ, LZD, CFZ, and DEL for the treatment of patients harboring MDR-resistant strains. This could explain why the rates of CFZ, BDQ, and DEL resistance (2.88, 2.88, and 1.04%, respectively) in our province were lower than those reported

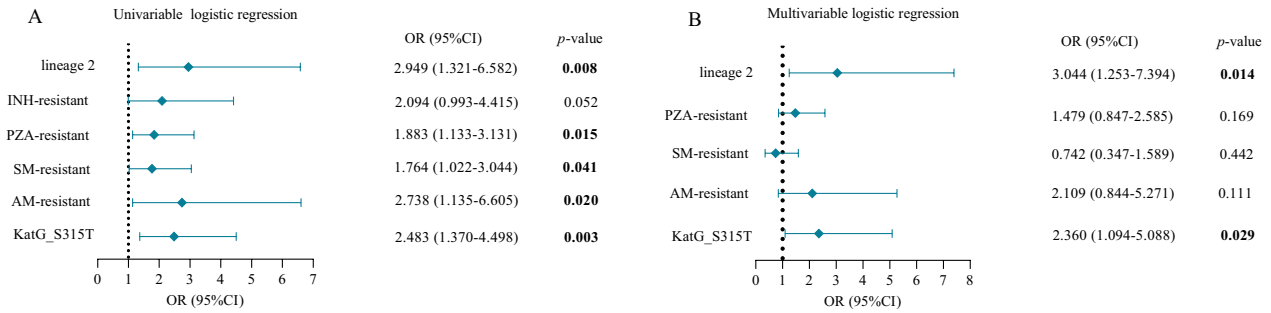

Fig. 5 Univariable and multivariable logistic regression analysis of risk factors for MDR/RR-TB transmission clusters. MDR/RR-TB, multidrug-resistant or rifampicin-resistant tuberculosis

nationally (6.65, 7.16, and 3.32%) [21]. Therefore, the use of new anti-TB drugs warrants standardization and the regular monitoring of resistance.

The genomic clustering rate of clinical TB strains reflects the local transmission of TB. In our study, the MDR/RR-TB clustering rate in Sichuan was 32.37% from 2019 to 2021, and among 233 (83.81%) patients, transmission probably occurred via MDR/RR-TB strains. The range of previously reported clustering rates of RR-TB or MDR-TB in China was 20.8–42.8% [6, 9, 11, 22–24]. Meanwhile, the reported transmission rates of RR-TB or MDR-TB elsewhere vary substantially by province in China, ranging from 37.1 to 73% [6, 22–24]. Differences in cluster rates across regions could be related to several factors, including the sample size and composition, study duration, and quality of local TB control programs [25, 26].

To identify the factors that facilitate MDR/RR-TB transmission, we analyzed demographic, bacteriological, and clinical factors using clustering. Here, only bacteriological factors, such as lineage 2 strains and KatG S315T, were associated with a higher risk of MDR/RR-TB transmission. Owing to their hypervirulence and wide geographic distribution, lineage 2 strains have emerged as important drivers of the global TB and MDR-TB burden and have a central role in the establishment of MDR epidemics in China [27–29]. In our study, 82.01% of MDR/RR-TB cases were lineage 2, similar to the rate reported among MDR-TB strains in China (87.4%) [29] and higher than that worldwide (70%) [30]. MDR/RR-TB strains belonging to lineage 2 also had a significantly higher risk of clustering (OR 3.044, 95% CI 1.253–7.394). Similarly, lineage 2 strains were reported to be associated with a higher risk of MDR/RR-TB transmission in previous studies in Shenzhen and Chongqing [11, 22].

MDR-TB strains are inherently weaker or less fit than drug-sensitive strains because harboring drug resistance-associated mutations is usually associated with fitness costs; however, this is not the case for strains with the RpoB S450L and KatG S315T variants [28, 31, 32]. We analyzed the association between these bacterial fitness-related mutations and transmissibility of the MDR/RR-TB strains. Strains with KatG S315T had a higher probability of genetic clustering (OR 2.360, 95% CI 1.094–5.088), whereas RpoB S450L was not associated with strain clustering ($\chi2=0.255$, $P=0.613$). Chen et al. [30] found that KatG S315T is significantly associated with the risk of MDR-TB clustering in developing countries, countries with highly MDR-TB-burdened, and lineage 2-dominant countries but that RpoB S450L is not significantly correlated with MDR-TB transmission.

Several risk factors for MDR-TB clustering have been identified in other studies, including patient age, occupation, and census registry [6, 11, 24]. Our study showed, little difference in clustering rates among the age groups. Despite strains from students and faculty (46.67%, 7/15) having a higher clustering rate than those from others (31.54%, 82/260), no significant association between occupation and the clustering rate was found ($\chi2=1.483$, $P=0.350$). Transmission between residents and migrants also had little impact on the recent transmission of MDR/RR-TB.

In terms of strain clustering, we collected the addresses of patients to describe their geographic distribution. In total, 66.67% (26/39) of the patients lived in the same county, indicating that recent transmission had mainly occurred in the county areas, similar to the results of a study in Hunan [9]. In addition, we identified the accumulation of new resistance-associated mutation sites along the transmission chain, especially for resistance to EMB, FQs, and PZA, indicating that MDR/RR-TB was not fully controlled [22]. In contrast, the descendant strains lost some drug-resistance mutations compared with the ancestral strains along the transmission chain. Possible reasons for this include differences in the latent phase of disease in individuals after transmission events and the fact that our study did not include all patients within the chain of transmission [33, 34].

Our study had some limitations. First, the proportion of recent transmission events might have been underestimated because only cases with culture-positive MDR/RR-TB over a 3-year period were included, and culture-negative MDR/RR-TB patients were not assessed. Second, the lack of information on the HIV status of patients precluded an analysis of correlations between HIV and genetic clustering. Third, owing to the absence of information about social characteristics, the history of active TB contact, and the venues frequently visited where transmission might have occurred, we could not determine the epidemiologic associations among patients based on MDR/RR-TB clusters.

Conclusions

In summary, transmission plays a significant role in the burden of MDR/RR-TB in Sichuan province. Targeted interventions, such as the early detection of cases, effective therapy, and patient management, should be implemented to reduce MDR/RR-TB transmission. Moreover, lineage 2 strains dominated in terms of the prevalence of MDR/RR-TB in Sichuan and increased the risk of its transmission, as did strains with the KatG S315T variant. Therefore, future studies should focus on these risk factors. Although resistance to FQs has substantially declined compared to that reported in our earlier study, drug resistance to FQs and PZA is still serious, warranting urgent DST prior to the design of therapeutic regimens. New anti-TB drugs, such as BDQ and LZD, must be used as the standard and regularly monitored for resistance.

Abbreviations

AM	Amikacin
BDQ	Bedaquiline
CFZ	Clofazimine
CI	Confidence interval
CM	Capreomycin
DEL	Delamanid
DR-TB	Drug-resistant tuberculosis
DST	Drug susceptibility testing
EMB	Ethambutol
ETO	Ethionamide
FQ	Fluoroquinolone
INH	Isoniazid
KM	Kanamycin
LZD	Linezolid
MDR/RR-TB	Multidrug-resistant or rifampicin-resistant tuberculosis
OR	Odds ratio
PAS	Para-aminosalicylic acid
pre-XDR-TB	Pre-extensively drug-resistant
PTB	Pulmonary tuberculosis
PZA	Pyrazinamide
SM	Streptomycin
TB	Tuberculosis
WGS	Whole-genome sequencing
XDR-TB	Extensively drug-resistant

Supplementary Information

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Additional file 1.

Additional file 2.

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Author contributions

WG: conceptualization, methodology, formal analysis, investigation, writing—original draft preparation, writing—review and editing; JH: conceptualization, methodology, formal analysis, investigation, writing—review and editing; SZ: performed experiments; WW: performed experiments; YG: performed experiments; HL: performed experiments; LH: performed experiments; JL: data analysis; TL: data analysis.

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Data availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA014982) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval

The study protocol was approved by the Ethical Review Committee at the Biomedical Ethics Committee, Sichuan Center for Disease Control and Prevention (No. 2024-001). All participants provided written informed consent after reviewing the description of the study.

Consent for publication

All authors no conflicts.

Competing interest

The authors declare no competing interests.

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