Transmission of multidrug-resistant *Mycobacterium tuberculosis* in Wuhan, China
A retrospective molecular epidemiological study

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Abstract

How multidrug-resistant tuberculosis (MDR-TB) spreads and expands in Wuhan population is not clear. The study aimed to determine the transmission patterns of MDR-TB in Wuhan city, China, including 149 patients with MDR-TB.

Tuberculosis isolates were genotyped by deletion-targeted multiplex polymerase chain reaction, mycobacterial interspersed repetitive unit-variable number tandem repeat typing, and sequencing of drug resistance-associated genes. The risk factors of genomic-clustering were analyzed with logistic regression. The genomic-clustering patients were deeply investigated.

The analysis identified 111 unique and 11 clustered genotypes (38 isolates). The clustering rate was 25.50% and the minimum estimate proportion of recent transmission was 18.12%. Two clusters (5 isolates) shared the same mutation, the remain 9 clusters (33 isolates) had different mutation. Logistic regression showed that older than 60 years (adjusted OR 2.360, 95% CI:1.052-5.292) was an independent factor associated with the genomic-clustering of MDR-TB. Among the 38 genomic-clustering cases, 14 cases had epidemiological transmission links. The most common type of transmission link was social contact.

The local transmission of MDR-TB in Wuhan was really an issue. The elderly population might be the high-risk groups for transmission of MDR-TB, and the community or public transportation might be the main transmission places.

Abbreviations: DTM-PCR = deletion-targeted multiplex polymerase chain reaction, HGDI = Hunter-Gaston discriminatory index, MDR-TB = multidrug-resistant tuberculosis, MIRU-VNTR = mycobacterial interspersed repetitive unit-variable number tandem repeat typing, MTB = *Mycobacterium tuberculosis*, RR-TB = rifampicin drug resistant tuberculosis, TB = tuberculosis, WGS = whole-genome sequencing.

Keywords: genotype, multidrug-resistant tuberculosis, *Mycobacterium tuberculosis*, transmission pattern

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1. Introduction

According to the World Health Organization global tuberculosis report,[1] 3.3% of new tuberculosis (TB) cases and 17.7% of previously treated TB patients were MDR/rifampicin drug resistant tuberculosis (RR-TB) (multidrug resistant tuberculosis [MDR-TB]; drug resistant tuberculosis, RR-TB) in 2019. It is estimated that 465,000 cases were RR-TB and 78.0% of them were MDR-TB. China has the second largest proportion (14.0%) of drug-resistant TB, and MDR-TB brings great challenges to tuberculosis control in China.

Although previous studies have explored the genetic diversity of MDR-TB isolates in China,[2–5] few studies combined the genotyping and epidemiological investigation on the transmission of MDR-TB. Yang et al[6] explored the transmission dynamics of MDR-TB in Shanghai, China, and concluded that transmission of MDR-TB had an important role in the burden of MDR-TB. Jiang et al's study[7] found that local transmission of MDR-TB was a serious problem in Shenzhen, China, and suggested schools and factories are common places of transmission occurrence. Wuhan, where our study took place, is the largest land, water and air transport hub in China's inland, and known as "the thoroughfare of nine provinces". Its unique geographical advantages also mean that this area is densely populated and mobile, which undoubtedly creates an ideal environment for the spread and epidemic of MDR-TB. How multidrug-resistant tuberculosis spreads and expands in Wuhan population is not clear.

In order to comprehensively understand the transmission level and pattern of MDR-TB in Wuhan, we combined 24-locus mycobacterial interspersed repetitive unit-variable number

tandem repeat typing (MIRU-VNTR) and deletion-targeted multiplex polymerase chain reaction (DTM-PCR) to genotype the isolates. Rifampicin resistance determining region of *rpoB* gene and isoniazid resistance-related genes (*katG*, *inhA*, *fabG* gene) were also sequenced to cluster. Furthermore, the genomic-clustering subjects were interviewed to explore the transmission dynamic of MDR-TB.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Wuhan Pulmonary Hospital Medical Ethics Committee (protocol number: 2018-12). All the patients signed an informed consent form before they participated in this study.

2.2. Subjects enrollment and drug susceptibility testing

In 2017, a total of 341 MDR *Mycobacterium tuberculosis* (MTB) were detected from 6072 active pulmonary tuberculosis cases registered in Wuhan TB reporting network, which belongs to the National Tuberculosis Program system. Among them, 151 cases were detected by Wuhan Pulmonary Hospital, which is 1 of the 2 designated hospitals for tuberculosis in Wuhan. After excluding 2 lost isolates, 149 isolates were included in this study.

MDR-TB was defined as being resistant to at least 2 primary first-line anti-TB drugs (rifampicin and isoniazid) in vitro.[8] The standard strain H37Rv was provided by the National Tuberculosis Reference Laboratory as the control strain in this experiment. MDR-TB isolates were cultured according to the national standardized rules for tuberculosis bacteriological test and stored in the strain bank at −80°C. MDR-TB isolates were confirmed by proportional drug susceptibility test with the following concentration: isoniazid (0.2 μg/mL), rifampin (40.0 μg/mL), streptomycin (4.0 μg/mL), ethambutol (2.0 μg/mL).

2.3. DNA extraction

Genomic DNA of the isolate was extracted according to the bacterial genomic DNA extraction kit provided by Daan Gene Biology Co., Ltd.

2.4. MIRU-VNTR typing

MIRU-VNTR typing was performed according to the international standard 24 loci method.[9] The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Each locus amplification reaction was performed in a total volume of 20 μL consisting of 10 μL 2 × Taq PCR MasterMix (CWBIO), 2 μL 2 μmol/L each primer, 1 μL DNA lysate and 5 μL ddH$_2$O. The PCR products were analyzed in a 1.5%~2.0% agarose gel using a 100 bp DNA ladder as the molecular weight standard. H37Rv and H$_2$O were used as positive and negative controls, respectively, in each PCR reaction. The number of tandem repeats was calculated based on the length of the repeat and flank sequences for each locus.

2.5. Deletion-targeted multiplex polymerase chain reaction

DTM-PCR was used to detect the deletion of RDl05 fragment to differentiate Beijing family strains. The modern or ancient

sublineage of Beijing family was identified by detecting the IS6110 in the NTF region. The detail of this method was described in Khosravi AD's[10] and Wada T's study.[11]

2.6. Drug resistance-associated genes sequencing

Drug resistance-associated genes sequencing was used for secondary genotyping of after the isolates were clustered by MIRU-VNTR and DTM-PCR. The following major resistance-related gene regions were directly sequenced: rifampicin resistance determining region of *rpoB* gene and isoniazid resistance-related genes (*katG*, *inhA*, and *fabG*).

2.7. Interview and epidemiological transmission links analysis

The clinical variables and demographic information of each MDR-TB case were derived from electronic medical records. Patients whose isolates were identified as genomic-clustered were invited for a questionnaire survey. The social relations, medical history, contacts with active TB patients and their commonly acting places was included in the survey. The epidemiological transmission links were defined as the following: confirmed epidemiological transmission link (patients knew each other or shared the same address or complex), probable transmission link (patients did not know each other but the distance of residence less than 2 km, or shared the same bus line in similar time period, or visited the same food market), and without transmission link (patients did not know each other and lacked a common neighborhood, setting or bus line).

2.8. Data analysis

The gel images of different MIRU-VNTR loci and their background information of each isolate were input into the computer, and the PCR amplification fingerprints of the tested isolates were compared with H37Rv. The molecular weights and repetitions of different MIRU locus of TB were calculated based on the repeated H37Rv sequences.

The online tool at website http://www.MIRU-VNTRplus.org was used to perform the clustering analysis.[12] We constructed minimum spanning tree by unweighted pair group method with arithmetic mean. The Hunter-Gaston discriminatory index (HGDI) values of 24 loci was calculated by the equation of Hunter and Gaston.[13] The discriminatory power of each locus was judged as poor if HGDI<0.3, moderate if 0.3≤ HGDI≤0.6, or high if HGDI>0.6.

The MTB isolates were considered to be clustered if they had identical MIRU-VNTR and DTM-PCR genotype.[14] The minimum estimate of the recent infection rate was calculated as follows: $(n_c − c)/n$, where n_c represents the clustered isolates, c represents the number of clusters, and n represents all samples of isolates.

SPSS 21.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. The demographic and clinical features of patients were compared between modern-Beijing and ancient-Beijing genotypes, and between clustered and non-clustered groups were assessed with univariate logistic regression. To analyze the influencing factors associated with the genomic-clustering, the multivariable logistics regression model was used. A forward, step-wise approach was used to select the variables.

Table 1

Demographic and clinical features of patients infected with Modern-Beijing and Ancient-Beijing genotype isolates.

	Number of Ancient Beijing family (n = 17)	Number of Modern Beijing family (n = 125)	OR	95% CI	*P* value
Sex					
Male	10	93	1.000		
Female	7	32	2.034	0.715-5.791	.18
Age (yrs)					
14-60	14	97	1.000		
≥60	3	28	1.347	0.361-5.022	.66
Treatment history					
New case	11	80	1.000		
Retreated	6	45	0.970	0.336-2.798	.96
Positive sputum smear result					
No	6	31	1.000		
Yes	11	94	1.654	0.565-4.843	.36

3. Results

3.1. Demographic and clinical features

The relationships of age, sex, sputum smear results, and treatment history with Beijing sublineage (modern and ancient sublineage) of 149 patients were shown in Table 1. The relationship between the variables and Beijing sublineage did not show statistical significance ($P > .05$).

3.2. DTM-PCR

All 149 strains were divided into Beijing strain (142 strains) and non-Beijing strain (7 strains). Among Beijing strains, 125 strains were identified as modern-Beijing sublineage and 17 strains were ancient-Beijing sublineage (Table 1).

3.3. Mycobacterial interspersed repetitive unit-variable number tandem repeat typing

Allelic diversity of each MIRU-VNTR locus are displayed in Table 2. Two out of 24 loci (QUB11b, MIRU26) had the high discriminatory power, 6 loci (Mtub21, Mtub04, QUB26, MIRU10, Mtub39, MIRU24) had the moderate discriminatory power, and the remain loci had the low discriminatory power.

By 24 loci MIRU-VNTR, 111 unique genotypes and 11 clustered genotypes (38 isolates) from 149 isolates were identified, and the cumulative HGDI was 0.9943 (Table 3, Fig. 1). The clustering rate was 25.50% and the minimum estimate proportion of recent transmission was 18.12%. The largest cluster contained 7 isolates and the smallest cluster contained 2 isolates. Except the isolates from the 2 cases in cluster 11 were non-Beijing lineage, the others were Beijing lineage

Table 2

Allelic diversity of each MIRU-VNTR locus for MDR-MTB isolates in Wuhan, China (n=149).

VNTR alias	Allele number												HGDI	Conclusion[*]
	0	1	2	3	4	5	6	7	8	9	10	11		
QUB11b	1	3	3	5	6	46	70	14	1	0	0	0	0.6738	High
MIRU26	0	3	1	6	6	44	77	10	1	1	0	0	0.6393	High
Mtub21	0	3	1	6	33	93	10	2	1	0	0	0	0.5553	Moderate
Mtub04	0	0	5	52	88	4	0	0	0	0	0	0	0.5279	Moderate
QUB26	0	1	3	3	1	2	8	13	105	11	1	1	0.4861	Moderate
MIRU10	0	5	36	106	2	0	0	0	0	0	0	0	0.4333	Moderate
Mtub39	5	5	119	17	3	0	0	0	0	0	0	0	0.3444	Moderate
MIRU24	0	118	0	0	0	31	0	0	0	0	0	0	0.3272	Moderate
ETRA	0	0	2	18	124	5	0	0	0	0	0	0	0.2887	Poor
QUB4156	0	0	0	128	9	11	0	1	0	0	0	0	0.2495	Poor
MIRU31	0	0	3	8	131	5	1	1	0	0	0	0	0.2188	Poor
MIRU39	0	2	12	132	3	0	0	0	0	0	0	0	0.2042	Poor
MIRU40	0	1	8	134	2	3	0	1	0	0	0	0	0.1834	Poor
Mtub30	0	0	10	2	137	0	0	0	0	0	0	0	0.1452	Poor
ETRC	0	3	1	139	5	1	0	0	0	0	0	0	0.1231	Poor
MIRU16	0	2	5	141	1	0	0	0	0	0	0	0	0.0978	Poor
MIRU04	0	1	3	142	2	1	0	0	0	0	0	0	0.0856	Poor
MIRU27	0	0	4	144	1	0	0	0	0	0	0	0	0.0593	Poor
ETRB	0	2	145	2	0	0	0	0	0	0	0	0	0.0466	Poor
Mtub34	0	0	2	145	2	0	0	0	0	0	0	0	0.0466	Poor
MIRU02	0	3	146	0	0	0	0	0	0	0	0	0	0.0332	Poor
MIRU23	0	0	0	0	0	147	2	0	0	0	0	0	0.0201	Poor
MIRU20	0	1	148	0	0	0	0	0	0	0	0	0	0.0068	Poor
Mtub29	0	0	0	149	0	0	0	0	0	0	0	0	0.0000	Poor

HGDI = Hunter-Gaston discriminatory index, MDR-MTB = multidrug-resistant *Mycobacterium tuberculosis*, MIRU-VNTR = mycobacterial interspersed repetitive unit-variable number tandem repeat typing.
[*] HGDI>0.6, highly discriminatory; 0.3≤ HGDI≤0.6, moderately discriminatory; HGDI<0.3, poorly discriminatory.

Table 3

Cumulative HGDI of each MIRU-VNTR locus (n = 149).

VNTR alias	Individual HGDI	Number of patterns	Number of clusters	Number of clustered isolates	Clustering rate (%)	Number of isolates in each cluster	Cumulative HGDI
QUB11b	0.6738						
MIRU26	0.6393	26	19	142	95.30	2-39	0.8637
Mtub21	0.5553	43	21	127	85.23	2-30	0.9221
Mtub04	0.5279	62	22	109	73.15	2-22	0.9521
QUB26	0.4861	81	19	87	58.39	2-16	0.9711
MIRU10	0.4333	89	19	79	53.02	2-15	0.9769
Mtub39	0.3444	96	18	71	47.65	2-13	0.9817
MIRU24	0.3272	102	18	65	43.62	2-9	0.9881
ETRA	0.2887	107	17	59	39.60	2-9	0.9896
QUB4156	0.2495	108	18	59	39.60	2-9	0.9912
MIRU31	0.2188	111	17	55	36.91	2-8	0.9923
MIRU39	0.2042	112	17	54	36.24	2-8	0.9926
MIRU40	0.1834	116	14	47	31.54	2-8	0.9931
Mtub30	0.1452	118	14	45	30.20	2-8	0.9934
ETRC	0.1231	119	13	43	28.86	2-8	0.9935
MIRU16	0.0978	120	12	41	27.52	2-8	0.9936
MIRU04	0.0856	121	11	39	26.17	2-8	0.9937
MIRU27	0.0593	121	11	39	26.17	2-8	0.9937
ETRB	0.0466	121	11	39	26.17	2-8	0.9937
Mtub34	0.0466	121	11	39	26.17	2-8	0.9937
MIRU02	0.0332	121	11	39	26.17	2-8	0.9937
MIRU23	0.0201	121	11	39	26.17	2-8	0.9937
MIRU20	0.0068	122	11	38	25.50	2-7	0.9943
Mtub29	0.0000	122	11	38	25.50	2-7	0.9943

HGDI = Hunter-Gaston discriminatory index, MIRU-VNTR = mycobacterial interspersed repetitive unit-variable number tandem repeat typing.

(Table 4). The genotyping results from MIRU-VNTR and DTM-PCR were consistent.

3.4. Sequencing results of drug resistance-associated gene

RpoB, *katG*, *inhA*, and *fabG* genes were sequenced in genomic-clustered isolates, and their mutation characteristics are shown in Table 4. Among the *rpoB* gene mutations, 28 cases had a well-known mutation of Ser450Leu substitution, 1 case had Asn437Asp substitution, 1 case had Asp435Tyr substitution, 4 cases had Asp435Val substitution, 2 cases had His445Leu substitution, and 3 cases had His445Tyr substitution. One out of the 38 isolates had double mutations of *rpoB* gene. Among the *katG* gene mutations, 25 cases had the most common Ser315Thr substitution, 1 case had Trp191Arg substitution, 1 case had Ser315Asn substitution, and 11 cases had no mutation. There was no mutation in *inhA* gene of all 149 isolates. Among all 149 isolates, 8 isolates had *fabG* gene mutation of C. −15C >T, only 1 isolate had C. −8C > T, and the other isolates had no mutation.

Among the 11 clusters, 5 clusters shared the same *rpoB* gene mutations, all of which were the most common Ser450Leu substitution. In the largest cluster that contained 7 cases, 2 MDR-TB cases whose isolates had the same rare substitution of Asp435Val, and the distance between their residence was within 5 km. In the cluster 4, 2 cases had isolates with the same rare substitution of Asp435Val, and the 2 cases shared same bus line in 1 year before the diagnosis of MDR-TB. Among the 11 clusters, 4 clusters had the same mutation in *katG* gene, of which 3 clusters had the most common Ser315Thr, and the other cluster had the wild-type gene. In the largest cluster that contained 7 cases, 4 cases had isolates with the substitution of Ser315Thr, and

the other 3 cases had isolates with wild-type gene. In addition, all isolates showed wild-type *inhA* gene. Five out of 11 clusters had identical *fabG* gene mutations, all of which were wild-type gene. One out of 11 clusters had wild-type *inhA* gene and −8C>T, and the remain 5 clusters had wild-type *inhA* gene and −15C>T.

Besides, the gene mutation of isolates in 2 clusters (5 isolates) were totally same, and the other clusters contained at least 1 isolate with different mutation.

3.5. Epidemiological transmission links of clustered cases and risk factors of genomic-clustering

According to the epidemiological survey, 8 out of the 38 MDR-TB cases had confirmed epidemiological transmission links, and 6 cases had probable epidemiological transmission links, and the remaining had no epidemiological transmission links (Table 5). The most common type of epidemiological transmission link was social contact, such as visiting the same food market, taking the same bus line, and residence proximity. Only 2 cases had close family contact and 2 cases had nosocomial infection.

Table 6 shows the results of risk factors associated with genomic-clustering of MDR-TB. Using the multivariate analysis, we found that older than 60 years was the independent factors associating with the genomic-clustering (adjusted OR 2.360, 95% CI:1.052-5.292).

4. Discussion

The MDR-TB is still a serious public health issue in China. According to the National Baseline Survey of Drug-resistant Tuberculosis conducted in 2007 to 2008, the overall prevalence of MDR-TB was 8.3% in smear-positive tuberculosis patients
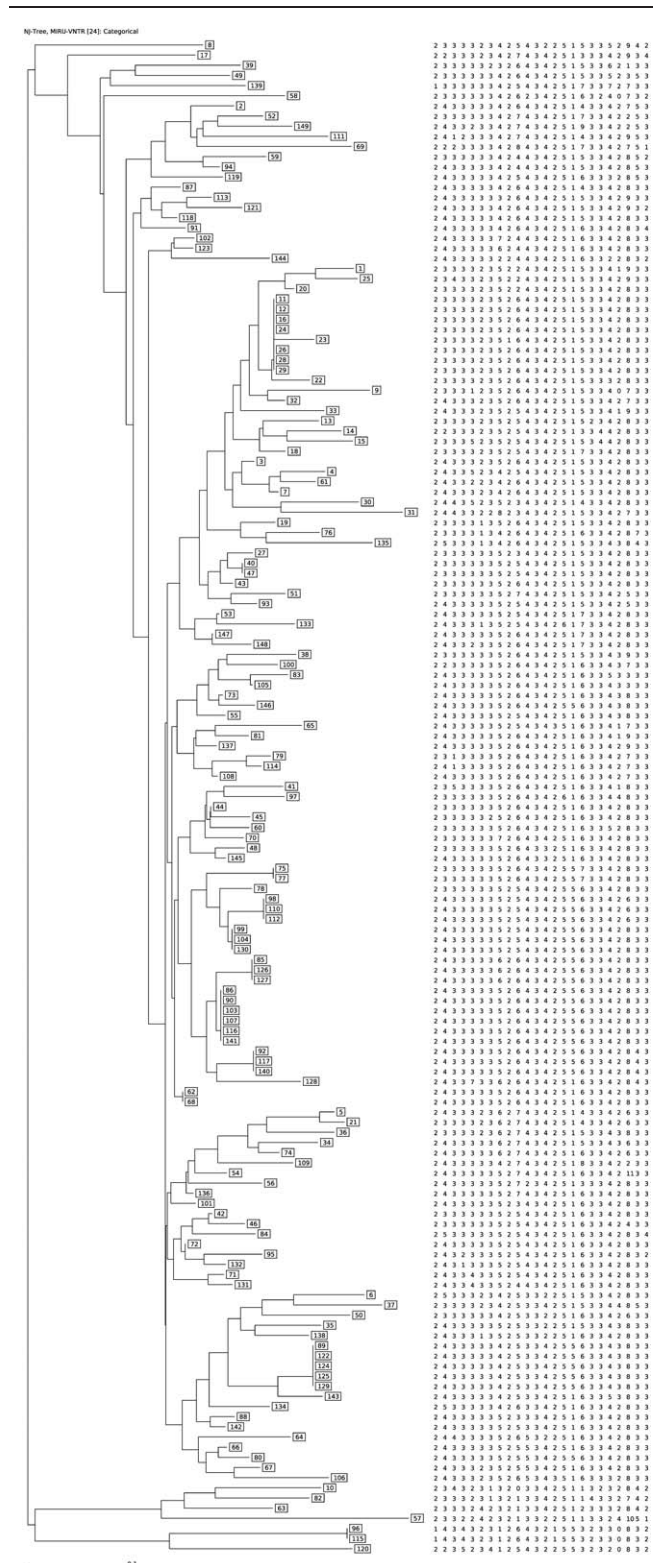
Figure 1. Minimum spanning tree of 149 MDR *Mycobacterium tuberculosis* isolates from Wuhan.

method and traditional epidemiological investigation to explore the transmission patterns of MDR-TB. The genotyping method included MIRU-VNTR, DTM-PCR and drug resistance-associated genes sequencing.

The genotyping of MTB plays an important role in tracing the source of infection in contact investigation,[16,17] distinguishing exogenous infection and endogenous relapse,[18] and outbreak investigation,[19,20] which can make up for the shortcomings of traditional epidemiological survey. Identification of Beijing family genotype is often the first step of genotyping MTB. The most widely used methods to detect Beijing family were spoligotyping[21,22] and DTM-PCR.[2] This study adopted DTM-PCR which was simpler and quickly. The second step of genotyping was RFLP[23] or MIRU-VNTR,[22] and MIRU-VNTR was more operational. The third step was whole-genome sequencing (WGS). However, because of the high cost and complex data analysis, sequencing of the drug-resistant gene was conducted as a substitute of WGS. In this study, we found that the HGDI of standard 24-locus MIRU-VNTR was relatively high, and the result of DTM-PCR genotyping remained unchanged. The further genotyping by sequencing of drug resistance-associated genes showed that several isolates in the same cluster shared the same rare mutation, indicated more chance to have the same transmission source.

National Baseline Survey of Drug-resistant Tuberculosis in China demonstrated that the proportion of drug-resistant patients who did not have treatment history was more than 70.0%. Shao's[18] study reported that 47.8% of treated patients with TB was due to reinfection rather than relapse. Several studies that combined WAS and traditional epidemiological methods showed the evidence of recent transmission of MDR-TB in China.[6,7] In the present study, we found that the clustering rate was 25.50%, the minimum estimate proportion of recent transmission was 18.12%, and the proportion of patients who had epidemiological transmission link accounting for 9.40%. The recent transmission rate observed in the present study may be underestimated. First, the MDR-TB patients in this study was the total patients in Wuhan Pulmonary Hospital, not included the patients in another designated hospital (Jin-yin-tan hospital), thus only representing about 40.0% of patients in Wuhan. Second, the proportion of culture-positive tuberculosis was only 40.6% (data in Wuhan TB reporting network) in Wuhan in 2017. Such a low proportion of culture-positive TB cases may reduce the effectiveness of molecular epidemiological studies. Third, WGS was more efficient in detecting epidemiological association than MIRU-VNTR.[24–26] Due to the high cost and complex analysis of WGS, we used MIRU-VNTR combing sequencing of common drug-resistant gene. Thus, the method rendered a suboptimal discrimination among different genotypes.

In this study, we found that the rate of epidemiological transmission link among genomic-clustered patients was lower than that observed in Yang et al's[6] study in Shanghai, whereas the result was similar to that in Jiang et al's[7] study in Shenzhen. Maybe the reason is that as well as in the present study, the subjects in the study in Shenzhen only represented about 40.0% of the whole MDR-TB cases. We also found the places where transmission most frequently occurred were community and public transportation. This was similar as Shanghai,[6] whereas it was different from Shenzhen[7] in which school and factory were the most common place transmission occurring. Maybe this difference is due to the proportion of inter-migrant in Shenzhen being much higher than Wuhan.

(5.7% among new cases; 25.6% among retreatment cases).[15] Wuhan city is the transportation hub of the central part and the fourth largest city in China. Based on the retrospective population-based study in Wuhan, we combined the genotyping

Table 4

Demographic and clinic characteristics of patients and genetic information of 38 MDR-MTB clustered isolates.

ID of cluster	Age	Sex	Previous treatment	MIRU-VNTR	Beijing	rpoB	katG	inhA	fabG
							Drug resistance gene mutation		
1	45	M	New	24333335254342425563342833	Yes	Ser450Leu	−	−	−15C>T
1	40	M	New	24333335254342425563342833	Yes	His445Tyr	Ser315Thr	−	−
1	22	M	Retreatment	24333335254342425563342833	Yes	Ser450Leu	Ser315Thr	−	−
2	60	M	Retreatment	24333335254342425563342633	Yes	Ser450Leu	Ser315Thr	−	−
2	60	M	New	24333335254342425563342633	Yes	Ser450Leu	Ser315Thr	−	−
2	78	M	New	24333335254342425563342633	Yes	Ser450Leu	Ser315Thr	−	−
3	68	M	Retreatment	24333336264342425563342833	Yes	His445Leu	Ser315Thr	−	−
3	59	M	Retreatment	24333336264342425563342833	Yes	Ser450Leu	Ser315Thr	−	−
3	14	F	New	24333336264342425563342833	Yes	Ser450Leu	−	−	−
4	61	M	New	24333335264342425563342833	Yes	Asp435Val	Ser315Thr	−	−15C>T
4	48	M	New	24333335264342425563342833	Yes	Ser450Leu	Trp191Arg	−	−15C>T
4	32	F	Retreatment	24333335264342425563342833	Yes	Ser450Leu	−	−	−15C>T
4	61	M	Retreatment	24333335264342425563342833	Yes	Asp435Val	Ser315Thr	−	−
4	22	F	New	24333335264342425563342833	Yes	Ser450Leu	Ser315Thr	−	−
4	47	M	New	24333335264342425563342833	Yes	Ser450Leu	Ser315Thr	−	−
5	28	F	Retreatment	24333335264342425563342843	Yes	Ser450Leu	Ser315Thr	−	−
5	51	M	Retreatment	24333335264342425563342843	Yes	Ser450Leu	Ser315Thr	−	−
5	88	F	New	24333335264342425563342843	Yes	Ser450Leu	−	−	−
6	51	M	Retreatment	24333335264342425163342833	Yes	Ser450Leu	−	−	−
6	61	M	New	24333335264342425163342833	Yes	His445Leu	Ser315Thr	−	−
7	61	M	Retreatment	23333235264342425153342833	Yes	Ser450Leu	−	−	−
7	63	M	Retreatment	23333235264342425153342833	Yes	Ser450Leu	−	−	−15C>T
7	45	M	New	23333235264342425153342833	Yes	Ser450Leu	Ser315Thr	−	−
7	37	M	Retreatment	23333235264342425153342833	Yes	Asp435Val	Ser315Thr	−	−
7	22	M	New	23333235264342425153342833	Yes	Ser450Leu	Ser315Thr	−	−
7	59	M	New	23333235264342425153342833	Yes	Asp435Tyr	−	−	−
7	41	F	New	23333235264342425153342833	Yes	Asn437Asp, Asp435Val	Ser315Thr	−	−15C>T
8	29	M	New	23333335254342425153342833	Yes	Ser450Leu	−	−	−15C>T
8	77	M	Retreatment	23333335254342425153342833	Yes	Ser450Leu	−	−	−
9	36	F	Retreatment	23333335264342425573342833	Yes	Ser450Leu	Ser315Thr	−	−8C>T
9	30	F	New	23333335264342425573342833	Yes	Ser450Leu	Ser315Thr	−	−
10	26	M	New	24333334253342425563343833	Yes	Ser450Leu	Ser315Thr	−	−
10	35	F	New	24333334253342425563343833	Yes	His445Tyr	−	−	−15C>T
10	73	M	Retreatment	24333334253342425563343833	Yes	Ser450Leu	Ser315Asn	−	−
10	72	M	New	24333334253342425563343833	Yes	Ser450Leu	Ser315Thr	−	−
10	35	M	Retreatment	24333334253342425563343833	Yes	His445Tyr	Ser315Thr	−	−
11	78	M	New	14343231264321553233830832	No	Ser450Leu	Ser315Thr	−	−
11	55	M	New	14343231264321553233830832	No	Ser450Leu	Ser315Thr	−	−

MDR-MTB = multidrug-resistant *Mycobacterium tuberculosis*, MIRU-VNTR = mycobacterial interspersed repetitive unit-variable number tandem repeat typing.

Table 5

Characteristics of multidrug-resistant tuberculosis genomic clusters (n=38).

	Number of cases in cluster	Number of cases living in the same district	Number of cases with positive sputum smear result	Number of new cases	Known risk factors (number of cases)	Epidemiologically linked	Nature of epidemiological link [number of cases]
Cluster 1	3	2	2	2	Retreatment (1)	No	
Cluster 2	3	2	3	2	Retreatment (1), old (1)[*]	Yes	Household [2]
Cluster 3	3	0	3	1	Retreatment (2), hospitalization (2)	Yes	Nosocomial (health center [2])
Cluster 4	6	2,2	3	4	Retreatment (2)	Probable	Social (bus [2], residence proximity [2])
Cluster 5	3	3	2	1	Retreatment (2), old (1)[*]	No	
Cluster 6	2	0	2	1	Retreatment (1)	No	
Cluster 7	7	2,2	7	4	Retreatment (3)	Yes	Social (community, food market [2])
Cluster 8	2	0	2	1	Retreatment (1), old (1)[*]	No	
Cluster 9	2	0	2	1	Retreatment (1)	No	
Cluster 10	5	0	2	3	Retreatment (2), Cook in shopping plaza (1)	Probable	Social (bus [2])
Cluster 11	2	0	1	2	Old (1)[*]	Yes	Social (former residence place [2])

[*] Older than 75 yrs old.

Table 6

Univariable analysis of risk factors associated with multidrug-resistance in genomic clusters.

	Non-cluster (n=111)	Cluster (n=38)	OR	95% CI	P value
Sex					
Male	80	29	1.000		
Female	31	9	1.249	0.531-2.936	.61
Age (yrs)					
14-60	89	24	1.000		
≥60	22	14	2.360	1.052-5.292	**.04**
Treatment history					
New case	73	22	1.000		
Retreated	38	16	0.716	0.337-1.521	.39
Positive sputum smear result					
No	29	9	1.000		
Yes	82	29	1.140	0.483-2.691	.77
Treatment outcome					
Cured or treatment completed	58	16	1.000		
Treatment failed	5	0	0.000	0.000	.99
Default, moved, or lost to follow-up	24	11	1.661	0.673-4.099	.27
Died	4	0	0.000	0.000	.99
Stop treatment due to drug-adverse-event	4	4	3.625	0.815-16.122	.09
unknown	16	7	1.586	0.557-4.516	.39
Beijing strains					
No	5	2	1.000		
Yes	106	36	1.178	0.219-6.337	.85

In addition, Yang et al[6] also investigated the risk factors associated with genomic-clustering. Consistent with their results, our observation also showed that older than 60 years was an independent risk factor of genomic-clustering. The elderly are more likely to lack knowledge about tuberculosis, which leads to serious delay in diagnosis or treatment.[27,28] This maybe a possible reason for high chance of transmission in the elderly. Therefore, elderly population deserved increased vigilance in MDR-TB control.

This study had several strengths. The subjects were the total MDR-TB cases detected by Wuhan Pulmonary Hospital, representing about half of the registered cases in Wuhan TB reporting network, thus this study is population-based, not hospital-based.

What is more, we combined molecular with traditional epidemiological method to explore the transmission pattern of MDR-TB in Wuhan city. The DTM-PCR, MIRU-VNTR, and sequencing of drug-resistance-associated genes were used for genotyping of MTB. The traditional epidemiological investigation was meanwhile conducted. Combining these 2 methods, we can better explore the population transmission dynamic of MDR-TB.

But this study had some limitations. MDR-TB patients in this study represented only about 40.0% patients in Wuhan. The sputum culture positivity rate of confirmed TB cases was relatively low (40.6%) in Wuhan in 2017, and the genotyping methods by this study had a suboptimal genotyping discrimination. Thus, the transmission level of MDR-TB in Wuhan might be underestimated.

Due to the retrospective study design, information such as the social relations, medical history, contacts with active TB patients and their commonly acting places among patients was based on the recall of the subjects, the recall bias cannot be avoided.

In summary, we concluded that the local transmission of MDR-TB in Wuhan was really an issue. The elderly population might be the high-risk groups for transmission of MDR-TB, and the community or public transportation might be the main transmission places. The findings of this study remind related department to take actions to prevent the transmission of MDR-TB.

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