Gene Mutation Characteristics and Cluster Analysis of Multidrug-Resistant Mycobacterium Tuberculosis in Urumqi

Jiandong Yang (✉ YJD3631@163.com)

Urumqi Centers for Disease Control and Prevention

Rayibai Yadikaer

Health and Family Planning Commission of Xinjiang Uygur Autonomous Region

Yanggui Chen

Urumqi Center For Disease Control and Prevention

Li Ma

Urumqi Centers for Disease Control and Prevention

Yaoqin Lu

Urumqi Centers for Disease Control and Prevention

Na Xue

Urumqi Centers for Disease Control and Prevention

Baolin Rui

Urumqi Centers for Disease Control and Prevention

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Abstract

Objective To understand the molecular biological characteristics of multidrug-resistant *Mycobacterium tuberculosis* in Urumqi by the analysis of gene mutation and clustering of multidrug-resistant *Mycobacterium tuberculosis* in Urumqi, to provide theoretical basis for the prevention and control of multidrug-resistant tuberculosis in the future.

Methods The tuberculosis patients who were diagnosed, registered, cultured positive *Mycobacterium tuberculosis* and over 16 years old in Urumqi were studied. Geno Type MTBDR plus Assay and MIRU-VNTR were used to analyze the gene mutation and clustering of multidrug-resistant *Mycobacterium tuberculosis* in Urumqi.

Result Mutation gene included Isoniazid resistance gene *katG* 315; Rifampicin resistance gene *rpoB* 526, 531; Ethambutol resistance gene *embB* 206; Streptomycin resistance gene *rpsL* 43 and RRs 1401; Ofloxacin resistance gene *gyrA* 74, 90, 91 and 94. It was shown that the Beijing genotype was predominant (49.530%) among these 319 strains. There were 14 strains multi-drug resistant strains, it was predominant (73.684%) of multi-drug resistant strains. Multi-drug resistant prevalence of Beijing strains was 8.861%. The clustering rate of MDR-TB was 5.263% in Urumqi.

Conclusion Molecular biological characteristics of multidrug-resistant *Mycobacterium tuberculosis* have some particularities in Urumqi. Therefore, modern molecular epidemiological techniques such as molecular biology and DNA fingerprinting can be used to improve the speed of diagnosis and to carry out case tracking and source investigation.

Introduction

Tuberculosis (TB) is a chronic infectious disease and it is called "white plague" with the largest number of human deaths [1]. At the same time, it is still the main killer endangering human life. According to the Global Tuberculosis Report of 2018, it is estimated that in 2017 there will be about 10 million new TB cases worldwide, the incidence of TB will be 133/100,000, the global estimated number of TB deaths will be about 157,000, and the mortality rate will be 17/100,000. China is one of the 22 countries who with high TB burden in the world, it is the third just followed India and Indonesia in the world. In recent years, the emergence of drug resistance tuberculosis (DR-TB) worldwide, especially multi-drug resistant tuberculosis (MDR-TB) posed a serious challenge to global TB prevention and control. The World Health Organization (WHO) estimated that 3.6% of new patients and 17% of retreated patients worldwide in 2017 will be multi-drug resistant tuberculosis or rifampicin resistant tuberculosis (MDR/RR-TB) patients [2].

China was one of the most serious disaster areas of MDR-TB. The rate of MDR-TB has been increasing in recent years [3]. The results of the fifth national tuberculosis epidemiological sampling survey in 2010 showed that: The total multi-drug resistance rate is 6.8%. The initial multi-drug resistance rate is 5.4%. The acquired multi-drug resistance rate is 15.4% [4]. Studies showed that there are significant differences in the distribution of MDR-TB in different regions [5]. Xinjiang as one of serious TB disaster area in China, the epidemic situation of MDR-TB is also not optimistic. The results of the fifth tuberculosis epidemiological sampling survey in Xinjiang Uygur Autonomous Region showed that the total multi-drug resistance rate was 1.16% [6]. Although lower than the national level, but from the incidence trend was more serious [7]. From this view, MDR-TB has become a major public health problem in China and even in the world, that need attach great attention.

With the increasing of MDR-TB global epidemic, the research on the influencing factors of MDR-TB was also deepening studied by many countries. Studies showed that the incidence of MDR-TB is related to gene mutation of *Mycobacterium tuberculosis* (MTB), socio-economic factors, living standards, living conditions, regional health resources allocation and service quality, meteorological factors, geographical factors, government support, implementation of TB strategy and TB management. Gene mutation is related to social and ecological factors, which is the result of the interaction of many factors in recent years [8-11], researchers focused on basic bacteriological and molecular epidemiological studies of MTB.

The results of foreign studies showed that Beijing family type was the most dominant genotype of multi-drug resistant MTB^[12]. Compared to other types, Beijing family type was more likely to cause MDR-TB in patients worldwide. The second was H3 type. Its risk of MDR-TB was only lower than Beijing type, but significantly higher than the other TB types^[13]. The results were similar to foreign studies. Beijing type was the main pathogenic type^[14–15]. Some scholars have suggested that this may be related to the virulence and infectivity of MTB strains with Beijing family genotype^[12]. In addition to the influence of strain genotype on the pathogenesis of MDR-TB, mutation of drug resistance gene locus of MTB is one of the most important factors lead drug resistance. Recent studies have shown that the common resistance genes of isoniazid were *katG* and *inhA*, rifampicin was *rpoB*, ethambutol was *embB*, streptomycin was *rpsL* and ofloxacin was *gyrA*. Among them, *katG*, *inhA* and *rpoB* were the most frequent in MDR-TB patients^[16–17]. The highest mutation rate of isoniazid resistance gene *katG* was 315 sites, and the highest mutation rate of *inhA* gene was 15 sites. But the highest mutation site in *rpoB* of Rifampicin was 531, 526 and 516. At the same time, some researchers said that there may have joint mutations between drug-resistant genes^[18–20]. In addition, molecular epidemiology is also expanding the scope of research, such as gene clustering analysis in the study of strain homology, DNA fingerprinting technology in the MDR-TB outbreak, epidemiological investigation and so on.

In this study, molecular epidemiological methods were used to understand the molecular epidemiological characteristics of MTB and its impact on the incidence of MDR-TB in the market.

Material And Methods

1 Mycobacterium tuberculosis isolates

The survey was conducted in eight tuberculosis designated hospitals in the city from January 2014 to March 2015. The eight designated hospitals provide services to all TB patients in Urumqi. 319 tuberculosis patients were enrolled in this study. In addition, sputum samples were collected from patients at different time points (point, morning and evening). Acid-fast staining and Roche culture were used for microscopic examination and isolation of MTB.

2 Drug susceptibility testing

All MTB isolates were tested by proportional method on Roche susceptibility medium (DST). The drug concentration was isoniazid (0.2 g/ ml), Li Fuping (40 g/ml), streptomycin (4 g/ml), ethambutol (2 g /ml), ofloxacin (2 g /ml), kanamycin (30 g/ml), (40 (g /ml) and curreomycin (40 g/ml). The identification of MTB strains was carried out by using the identification medium of MTB.

3 DNA extraction and sequencing

The primers used in this study were obtained from refer to literatures. The primers were synthesized by "Hua Da Gene". DNA of MTB was extracted by ultrasonic lysis. Specific methods as follows: 500 ml sputum specimens added into 1.5 ml centrifugal tube; centrifugal for 5 minutes at 12 000 r/min, discarding supernatant, adding 500 ml sterilized water, swirling suspension precipitate, 12 000r/min centrifugation for 5 minutes, discarding supernatant, adding 100 ml sterilized water to each tube, swirling suspension; incubation specimens at 95°C for 20 minutes in water bath, ultrasonic decomposition for 15 minutes, and 12 000 r/centrifugation for 5 minutes. Minutes later, the supernatant was transferred to a new 500 ml centrifugal tube and stored at – 20°C for reserve. The extracted DNA was amplified. Finally, the PCR products were purified and sequenced on 3730 DNA analyzer.

4 Genotyping method

MIRU02, MIRU04, MIRU10, MIRU16, MIRU20, MIRU23, MIRU24, MIRU26, MIRU27, MIRU31, MIRU39 and MIRU40 were selected according to the bacterial gene database. There were 12 tandem repeat loci. The amplified product of 5 µl PCR

was added with 1 µl sample buffer, placed in the sample pore, covered with electrophoresis cell and connected with power supply. The electrophoresis was carried out by 1–5 v/cm voltage drop (calculated by the distance between the two poles). Finally, the ultraviolet gel imaging system was used for image preservation.

5 Analysis method

The data were analyzed by χ^2 test, Fisher exact probability test and cluster analysis. The software used Stata 14.0 package and cluster analysis online MIRU-VNTRplus statistical analysis tool(<http://www.miru-vntrplus.org>).

Standards

1 Diagnostic criteria for MDR-TB

Diagnostic criteria of tuberculosis cases refer to *diagnostic criteria for health industry standard of People's Republic of China (WS288-2017)*.

2 Inclusion criteria

- (1) Registered by the municipal tuberculosis prevention and control agency in Urumqi;
- (2) Diagnosis as TB cases refer to *diagnostic criteria for health industry standard of People's Republic of China (WS288-2017)*.
- (3) TB patients with initial treatment or retreatment;
- (4) MTB strains;
- (5) Mycobacterium positive.

3 Exclusion criteria

- (1) Whose age under 16 years;
- (2) Who don't agree to participate in this survey;
- (3) Who with severe complications.

4 Standards of quality control

- (1) Strictly abide by the Laboratory Biosafety Manual to ensure participants safety.
- (2) Testing should be strict accordance with the test operation steps stipulated of the national or industrial standards to avoid measurement bias caused by different test conditions and procedures.
- (3) The reagents and safety protective devices used in the test were all in effective use, otherwise will be discarded. All the equipment and instruments required in the test shall be affixed with the qualified green label of the national quality inspection department and the metrology department, and within the validity period, the systematic bias caused by the instrument and equipment shall be avoided.

Results

1 Distribution of mutation drug resistance genes

Resistance genes and locus of strains that multidrug-resistant MTB was tested. The result show that only katG gene is mutation in resistance genes of isoniazid (INH), and the mutation have significance compared with the group of sensitive to all drug ($P \leq 0.05$). The mutation locus of resistance gene katG is 315 locus, and base pair AGC has changed to AAC or

ACC, and amino acid serine (Ser) has changed to asparagine (Asn) or threonine (Thr). Resistance gene *ropB* of rifampicin (RFP) has mutation, and the mutation locus are 526 and 531. The base pair CAC of locus 526 and base pair TCG of locus 531 have changed to TAC and TTG respectively, and corresponding histidine (His) and serine (Ser) has changed to tyrosine (Tyr) and Leucine (Leu) respectively. It is significance compared with group of sensitive to all drug ($P \leq 0.05$). In aspect of ethambutol (EMB), resistance gene *embB* has mutation, and the mutation locus is 206. The base pair ATG has changed to ATA and GTG. However, compared with group of sensitive to all drug, base pair ATG changed to GTG is not significance ($P = 0.119$). In aspect of streptomycin (SM), resistance gene *rpsL* and *rrs* have mutation, and the mutation locus are 43 and 1401 respectively, and base pair AGG and A have changed to AAG and G respectively. Moreover, corresponding Arginine (Arg) and alanine (Ala) have changed to lysine (Lys) and glycine (Gly) respectively. Compared with group of sensitive to all drug, these mutation are significant difference ($P \leq 0.05$). The mutation locus of resistance gene *gyrA* in ofloxacin (OFX) have four. These locus are 74,90,91 and 94. With the exception of 90 locus, the mutation of other locus have both significant difference ($P \leq 0.05$). In addition, we found also that there is not mutation of resistance gene in capreomycin (CMP), kanamycin (KAM) and amikacin (AMK). (see Table 1)

Table 1
The mutation characteristics of resistance gene in multi-drug resistant MTB

Drugs	Gene	Locus	Mutation	Amino acid	Multi drug(<i>n</i> = 19)		All sensitive(<i>n</i> = 254)		<i>P</i> *
					Mutant strains(<i>n</i>)	Mutation rates(%)	Mutant strains(<i>n</i>)	Mutation rates(%)	
INH									
	katG	315	AGC→AAC	Ser→Asn	3	15.789	0	0.000	≤0.001
	katG	315	AGC→ACC	Ser→Thr	14	73.684	0	0.000	≤0.001
	inhA	-	-	-	0	0.000	0	0.000	-
RFP									
	rpoB	526	CAC→TAC	His→Tyr	2	10.526	0	0.000	≤0.001
	rpoB	531	TCG→TTG	Ser→Leu	14	73.684	0	0.000	≤0.001
EMB									
	embB	206	ATG→ATA	Met→Ile	4	21.053	11	4.331	0.014
	embB	206	ATG→GTG	Met→Val	3	15.789	15	5.906	0.119
SM									
	rpsL	43	AGG→AAG	Arg→Lys	10	52.632	2	0.787	≤0.001
	rrs	1401	A→G	Ala→Gly	3	15.789	2	0.787	0.003
OFX									
	gyrA	74	GCC→TCC	Ala→Ser	1	5.260	0	0.000	0.070
	gyrA	90	GTC→GTG	Val→Val	1	5.260	1	3.393	0.135
	gyrA	91	TCG→CCG	Ser→Pro	2	10.526	0	0.000	0.005
	gyrA	94	GAC→AAC	Asp→Asn	3	15.789	0	0.000	≤0.001
	gyrA	94	GAC→GGC	Asp→Gly	1	5.260	0	0.000	0.070

2 The distribution characteristic of gene type

We had genotyping to multi-resistance drug MTB strains through method of 12 locus MIRU-VNTR gene type. The result show that the kind of gene type is four that Beijing type, T1 type, T2 type and Special type in multi-drug resistance MTB. And most of multi-drug resistance rate is Beijing type (8.861%), the next of Special type (7.500%), the last is T1 type (1.282%). (see Table 2)

Table 2
Genotype distribution of multi-drug resistant MTB

Gene type	Number (n)	Number of multi-drug (n)	Rates of multi drug resistant MTB (%)
Beijing type	158	14	8.861
T1 type	78	1	1.282
T2 type	21	1	4.762
Special type	40	3	7.500

3 Cluster analysis

After genotyping, we had cluster analysis on the basis of genotyping. We find that number of clusters is one, if we believe similarity above 95% is one cluster (see Fig. 1) .According to conclusions from above, if we assume that one strain in each cluster is infection source of the cluster, then we estimate that 5.263% [(2 – 1/19) of peoples who infect multi-drug resistant MTB are because of infected. The result show, when maximum complex trajectory difference equal to 2 (DL = 2), the optimal propagation path fitting of significance in multi drug resistant MTB is three group, which it is precondition that without consider other factor and only consider relationship between genes of multi drug resistant MTB. The three groups are complex1(ID of patient is 1071957222608880 and 204),complex2 (ID of patient is 591244 and 53),and complex3(ID of patient is 6 and 58). It is interesting that gene type is absolutely identical and the similarity distance is zero between patient 107 and patient 195. (see Fig. 1 and Fig. 2)

Discussion

At present, mutation of the first-line anti-tuberculosis drug resistance genes have been extensively studied at home and abroad, such as INH, RFP, EMB and SM. Studies showed that the mutation sites of the drug resistance genes of the above anti-tuberculosis drugs are quite different in different regions ^[21].This study showed that the mutation rates of isoniazid resistance gene katG, rifampicin resistance gene rpoB, ethambutol resistance gene embB, streptomycin resistance gene rpsL and RRs in multidrug resistance group were higher than those in full-sensitivity group, which consistent with the results of domestic or foreign research ^[22–25].However, there was no mutation in inhA gene of isoniazid-resistant group and full-sensitive group, which was inconsistent with the results of Asho Ali et al. The results showed that the mutation rate of inhA gene was about 8% ^[26]. This difference may relate with geographical differences. At the same time, the results showed that the mutation site of INH resistance gene katG was 315. The base pairs of INH resistance gene katG were converted from AGC to AAC and AGC to ACC, while the amino acids were converted from Ser to Asn and Thr, this result consistent with Mekonnen D et al ^[27]. Studies showed that the anti-tuberculosis mechanism of INH is mainly to inhibit the synthesis of cell wall of MTB and make it lose its proliferative ability, which leads to the apoptosis of MTB ^[28]. In this process, the KatG gene of MTB (catalase peroxidase encoding gene) plays a key role. The above-mentioned anti-tuberculosis effect of INH could be accomplished only by converting it into its biological active isonicotinic acid under the action of catalase-peroxidase. The codon mutation at the 315 locus of katG gene resulted in the conversion of amino acids from serine to asparagine (Asn) and threonine (Thr), caused a change in the protein structure encoded by the katG gene, leaded a decrease in catalase peroxidase activity, which ultimately prevented the conversion of isoniazid to its bioactive isoniazid, resulted in the loss of isoniazid resistance ^[29]. Because there was no inhA gene mutation in this study, there was no combined mutation of katG gene and inhA gene, which resulted in isoniazid resistance in MDR-MTB. This

could provide some reference to future genetic diagnosis of MDR-TB in Urumqi. The highest mutation rate of RFP resistance gene *rpoB* was 531. The base of RFP was transformed from TCG to TTG, and the amino acid was transformed from Ser to Leu. This was consistent with the results of Nwofor AC and Hu Qiong et al ^[30]. In terms of mechanism, serine at the 531 site of *rpoB* gene was the most critical target for RFP to act on MTB. Once mutation occurs, it will directly lead RFP resistance in MTB. At the same time, the mutation at 526 site of *rpoB* gene also occurred in this study, suggesting that there may be a joint mutation at 526 site and 531site. The *embB* mutation of the EMB resistance gene was 206; the base group was mainly converted from ATG to ATA, and the amino acid was converted from Met to Ile. This was inconsistent with Khosravi AD et al.'s research, which showed that 306 site is a common mutation site of *embB* of the EMB resistance gene ^[31]. It was suggested that the *embB* gene of multi-drug resistant MTB may have some regional specificity, which can be used as a reference site for gene screening and rapid diagnosis of drug-resistant TB in Urumqi. The results are consistent with those of Smittipat N, Rezaei F and others ^[32–33]. The results showed that the mutation sites of *rpsL* and *RRs* of SM resistance gene were 43 and 1401 ; the mutation bases of 43 sites were transformed from AGG to AAG, and the mutation base pairs of 1401 sites were transformed from A to G. The mutations of *rpsL* and *RRs* resulted in the encoding disorders of the glycosomal protein S12 and ribosomal 16SrRNA, respectively, leading to the resistance of MTB to SM, while the most common mutations of *rpsL* and *RRs* were 43 and 1401 ^[34]. It was also found that there were joint mutations in the SM resistance genes *rpsL* and *rrs*. Mutation analysis of drug resistance genes of second-line drugs showed that only *gyrA* gene of OFX resistance mutated in four second-line anti-tuberculosis drugs, and the mutation sites were 74, 90, 91 and 94. This is consistent with the results of Wang Z, Sirous M and others ^[35–36]. From the above analysis of drug resistance gene site we could see that there were not only the genetic and molecular biological characteristics of the mainstream MDR-TB strains at home and abroad, but also has a few special characteristics. These characteristics will provide a new idea for rapid detection of MDR-TB in Urumqi in the future. With the deepening of molecular biology research on multi-drug resistant MTB strains, it was also suggested that the specialized TB prevention and control institutions and specialized hospitals in Urumqi should make full use of modern MDR-TB diagnosis technology to make rapid and accurate diagnosis, so as to gain valuable time for follow-up treatment. According to the genotype distribution characteristics of MTB: In this study, 12-locus MIRU-VNTR genotyping method was used to genotype multidrug-resistant MTB in Urumqi. It was found that 19 strains of multidrug-resistant MTB could be divided into four genotypes, named Beijing type, T1, T2 and idiotype. Among them, the best potential genotype of multidrug-resistant MTB in Urumqi was Beijing type. This result consistent with Yuan L et al ^[37]. MTB with Beijing genotype has the characteristics of rapid transmission, strong virulence and wide epidemic range. According to the results of genotyping, the dominant strain of MDR-TB in Urumqi is Beijing, which give a new challenge to the prevention and control of MDR-TB in Urumqi. It also suggests that the disease prevention and control agencies should pay close attention to monitoring drug resistance (especially RNH and RFP) in the treatment and management of TB patients whose strain genotype is Beijing in the future, and adjust the treatment plan in time to avoid the deterioration of the disease and the multi-drug resistance of MTB. This study also found that the multidrug resistance rate of the idiotype was second just behind Beijing type. This phenomenon may be caused by the large floating population and frequent population flow in our city, leaded a wide range of genotypes of MTB strains, and the idiotype produced by genotype-related hybridization of each strain. This idiotype retains the predominant resistance characteristics of the original genotypes, which make it easier for the idiotype to produce multi-drug resistance. In terms of clustering, MIRU-VNTR technique at 12 locus was used to cluster 19 strains of MTB isolated from multidrug-resistant patients. The results showed that 2 of 19 MDR-TB patients could be clustered into one cluster. It was inferred that the homology of MDR-TB in Urumqi is 5.263%, which is slightly lower than similar studies ^[38]. This may be related to the implementation of the new Trinity TB management model in our city since 2012. The Trinity TB management model refers to the establishment of a clear division of labor and coordinated service system for TB prevention and control agencies, designated medical institutions and grass-roots medical and health institutions. The construction of this prevention and control system greatly improved the efficiency of TB from prevention, diagnosis, treatment to management, and could discover the source of infection in time, control it at the same time, effectively blocking the spread of tuberculosis. Under this model, the incidence of MDR-TB and the probability of homologous

transmission of MDR-TB could be reduced [39]. As an effective modern TB management mode, the Trinity management mode should be implemented by the disease prevention and control agencies unremittingly. On this basis, combined with Urumqi TB epidemic situation, regional characteristics and other aspects of improvement, in order to adapt the actual needs of TB prevention and control work, greatly meet and ensure the actual interests of TB patients. Further analysis of the minimum spanning tree under complex paths showed that there were 3 optimal transmission paths for 19 MDR-TB patients. At the same time, combined with the results of genotyping, it was found that there might be a relationship between infection and infection between patients 107 and patients 195. By reviewing the questionnaires of patients No. 107 and No. 195, easy to find that they did not live in the same district and county, but the occupations of patients No. 107 and No. 195 were worker and farmers, which suggested that there may be employment relationship between them. The field epidemiological case study found that there was no social relationship between them, such as relatives, friends and colleagues, but there was employment relationship between them. At the same time, we know that No. 195 earlier infection TB at December 10, 2013, and the confirmation time was September 10, 2014. First time of No. 107 infection TB was September 2, 2014, and the confirmation time was September 19, 2014. Meanwhile, patients 107 worked in villages where patients No. 195 lived from January 2012 to September 2014, and patients No. 195 employed patients 107 during 2014. They had close contact with each other, mainly in farmland and the residence of patients No. 195. Two people recalled that the contact time between the two people in the residence of patient No. 195 was longer than that in the farmland, but the specific contact time could not be confirmed. We believe that patients 107 are more likely to be infected in the residence of patients 195. The preliminary success of the Etiology Survey provides relevant experience for the future investigation of TB outbreaks, especially MDR-TB outbreaks in our city. According to the latest reports, breakthroughs have been made in the application of molecular epidemiological methods such as gene fingerprinting, gene homology and cluster analysis in source tracking and source identification, and they have been gradually applied in practice, especially in source identification and chain tracking of major infectious disease outbreaks [40–41]. Moreover, this method had high specificity, high efficiency and high speed, which had won valuable time for rapid eradication of the epidemic situation [42]. Therefore, molecular epidemiological techniques should be further developed in the investigation of TB outbreaks in order to achieve scientific and rapid prevention and control.

At present, the study on molecular epidemic characteristics of multidrug-resistant MTB in Urumqi city level is still blank. This was the first report on the molecular biology of MDR-TB in Urumqi. At the same time, this study provided relevant experience for the future prevention and control of MDR-TB and outbreak investigation (mainly referring to the tracing of infectious sources) in Urumqi, and also provided reference for the research to other related infectious diseases. In addition, the results of this study will have great scientific value and social and economic effects on the prevention and control of MDR-TB in Urumqi. The study was influenced by time, long treatment cycle and low incidence of MDR-TB. In fact, only 319 eligible patients were surveyed in limited research time. Only 19 cases of MDR-TB were found. Although the sample size can basically meet the needs of analysis, the sample size was still insufficient. In the future, the investigation time can be prolonged appropriately, more MDR-TB cases can be collected, and more accurate data can be obtained. Secondly, although the results of this study were the same as those of the mainstream research at home and abroad, they also have their own characteristics. Because the research in this field in Urumqi is still blank, it could not provide evidence for this study. Therefore, further studies are needed to provide evidence for these specific results.

Conclusion

INH resistance gene *katG*315 mutation, RFP resistance gene *rpoB*526, 531 mutation, EMB resistance gene *embB*206 mutation, SM resistance gene *rpsL*, RRs corresponding 43, 1401 mutation and OFX resistance gene *gyrA*74, 90, 91 and 94 mutation; 12 MIRU-VNTR site genetic differences and typing. Differentiation was characterized by small genetic differences and high typing discrimination (HGI = 0.725); genotype distribution was characterized by Beijing type with the

highest rate of multi-drug resistance (8.861%); cluster analysis showed that the clustering rate of MDR-TB in Urumqi was 5.263%.

Declarations

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Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

Yang Jiandong (Department for Tuberculosis Control and Prevention, Urumqi Center for Disease Control and Prevention, Urumqi, Xiamen Road 18, 830026, Xinjiang, China. E-mail:YJD3631@163.com); Rayibai-Yadikaer(Health inspection of Health and Family Planning Commission of Xinjiang Uygur Autonomous Region, Urumqi, Longquan Road 191, 830001, Xinjiang, China. E-mail:420383531@qq.com); Chen Yanggui (Department for Tuberculosis Control and Prevention, Urumqi Center for Disease Control and Prevention, Urumqi, Xiamen Road 18, 830026, Xinjiang, China. E-mail:cdctb@126.com); Ma Li (Department for Tuberculosis Control and Prevention, Urumqi Center for Disease Control and Prevention, Urumqi, Xiamen Road 18, 830026, Xinjiang, China. E-mail:497976767@qq.com); Lu Yaoqin(Department of Occupational and Environmental Health, Xinjiang Medical University School of Public Health & Science and Education Department, Urumqi Center for Disease Control and Prevention, Urumqi, Xiamen Road 18, 830026, Xinjiang, China. E-mail:35278597@qq.com); Rui Baolin(Department for Tuberculosis Control and Prevention, Urumqi Center for Disease Control and Prevention, Urumqi, Xiamen Road 18, 830026, Xinjiang, China. E-mail:1441948546@qq.com)

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Figures

Figure 1

19 strains of multi-drug resistant strains of MTB gene phylogenetic tree

Figure 2

The minimum spanning tree complex paths Remark: We had analyzed the minimum spanning tree complex paths to gene type of 19 multi drug resistant Mycobacterium tuberculosis. Different color represents different gene type. The size of circle represents arise frequency of multi drug resistant Mycobacterium tuberculosis genotype. And the number on the line represent distance between difference gene type.