Biomedical Research 2017; 28 (10): 4594-4598 *ISSN 0970-938X*
www.biomedres.info

Multi-drug resistance of *Mycobacterium tuberculosis* strains in Tianjin, China from 2006 to 2015.

Zhang Li-Xia, Qin Zhong-Hua, Sun Hai-Bai, Wu Min, Wu Qi*

Department of Clinical Laboratory, Tianjin Haihe hospital, No. 890, JinGu highway, Tianjin, PR China

Abstract

Introduction: Drug resistance to *Mycobacterium tuberculosis* (*M. tuberculosis*) especially multi drug resistant tuberculosis (MDR-TB) has become a serious public health problem in China and worldwide. We retrospectively analysed the Drug Susceptibility Testing (DST) of 4 Tuberculosis (TB) drugs in 10 years, and provide reference for the prevention and treatment of tuberculosis.

Methods: 10856 strains were isolated from specimen of TB patients during 2006-2015 at our hospital; the liquid culture method was used for DST of 4 drugs-Streptomycin (S), Isoniazid (I), Rifampin (R) and Ethambutol (E). Analysis was performed for monodrug, polydrug and multi drug susceptibility testing.

Results: The total drug resistance rate was 33.92% (3682/10856); the total monodrug resistance rate was 13.38% (1452/10856); the total Polydrug Resistant (PDR) rate was 7.88% (855/10856) and the total Multidrug Resistant (MDR) rate was 12.68% (1375/10856). The single drug resistance resistant rate of Isoniazid (I) was higher than the other 3 anti-tuberculosis drugs every year. The single and multi-drug resistant rates to ethambutol were low. The Multidrug Resistance (MDR-TB) rate was higher in local region than national average.

Conclusion: Adjust the treatment plan of tuberculosis, strengthen the supervision and management are very necessary to control tuberculosis. More effective measures are still needed to curb the outbreaks and the recurrence rate of MDR-TB.

Keywords: Tuberculosis, Drug resistance, Multidrug-resistant, First-line drug.

Accepted on March 4, 2017

Introduction

Tuberculosis (TB) is a respiratory infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) and endangers human health. Currently, drug resistance to *M. tuberculosis* especially Multi Drug Resistant Tuberculosis (MDR-TB) has become a serious public health problem in China and worldwide [1-3]. According to the 2013WHO's report [4], high resistant and multiple drug resistant *M. tuberculosis* are important reasons of progression and increased mortality of TB. China is one of the most serious regions plagued by TB [5]. The fifth national TB epidemiological report of China [6] showed the number of patients with tuberculosis is 1300,000, which accounting for global prevalence of 14.3%; and ranked second in the world. There are 100,000 new MDR-TB patients were diagnosed every year and the MDR-TB patient has reach to 340,000 in China [7,8]. Recent reports showed the spreading of TB has the characters of high infection rate, high resistance rate, high morbidity, high mortality and low degradation rate [9-11].

MDR-TB does not respond to treatment with first-line drugs, and its management using second-line drugs has not yet been properly organized by most TB control programs [12,13]. Drug Susceptibility Testing (DST) of *M. tuberculosis* is a crucial procedure to determine the effective drug regimen for patients' treatment and could provide useful reference for the treatment of patients. In this study, we retrospectively analysed the10 years' DST of 4 first-line drugs to *M. tuberculosis* in, to provide reference for the prevention and treatment of tuberculosis.

Patients and Methods

Patients and samples

This study was approved by the Institutional Review Board of Tianjin Haihe hospital, and was conducted in accordance with good clinical practice. All applicable regulatory requirements and the guiding principles of the Declaration of Helsinki were abided. Informed consent was obtained from all subjects for using their samples.

The strains of *M. tuberculosis* were collected and isolated from the specimens of inpatient and outpatient in our hospital from 2006 to 2015. A total of 10856 *mycobacterium tuberculosis* positive strains were isolated. Four first line tuberculosis drugs: Streptomycin (S), Isoniazid (I), Rifampicin (R), Ethambutol (E) (all purchased from BD Biosciences, Franklin Lakes, NJ, USA) were used for DST.

Drug susceptibility testing (DST)

DST for all isolated strains was performed according to routine laboratory diagnosis of tuberculosis [14]. Briefly, the sputum samples were treated with 4% sodium hydroxide for digestion, and then the 0.5 ml of treated sample was added to the 7 ml MGIT culture tube containing medium and additives. The samples were cultured in BACTEC™ MGIT™ 960 Mycobacterial Detection System (BD Biosciences, Franklin Lakes, NJ, USA). Acid fast staining was performed in reported positive sample of MGIT™ 960 system. The DST was performed according to instructions of testing kits [12]. Isolates with inconsistent results were subjected to DST twice at different intervals to reproduce similar results. The concentrations of the drugs used were: 83 μg/ml for streptomycin (S); 8.3 μg/ml for isoniazid (I); 83 μg/ml for Rifampicin (R) and 415 μg/ml for Ethambutol (E). The standard strains of *M. tuberculosis* H37Rv was selected as quality control for each batch of test.

Definition of drug resistant M. tuberculosis

In this study, the drug resistance to *M. tuberculosis* was defined to according to WHO Guidelines for the programmatic management of drug-resistant tuberculosis (Emergency update 2008). Mono-resistance tuberculosis indicates *M. tuberculosis* is proved to be resistant to 1 first-line anti-tuberculosis drug in *in vitro* DST assay. Polydrug resistance tuberculosis indicates *M. tuberculosis* is proved to be resistant to more than 1 first-line anti TB drug (but resistant to isoniazid and rifampicin at the same time). Multi Drug Resistant Tuberculosis (MDR-TB) refers to tuberculosis *M. tuberculosis* is proved to be resistant to isoniazid and rifampin at least in *in vitro* DST test.

Results

There were 10856 strains of *M. tuberculosis* were isolated in 10 years. The drug resistance rate was 33.92% (3682/10856); the monodrug resistance rate was 13.38% (1452/10856); the Polydrug Resistant (PDR) rate was 7.88% (855/10856); the Multidrug Resistant (MDR) rate was 12.68% (1375/10856).

Drug resistant status

For different annual strains, the single drug resistant rate of Isoniazid (I) was higher than the other 3 drugs every year. The summarized frequency of single drug resistance: I (Isoniazid)>S (Streptomycin)>R (Rifampicin)>E (Ethambutol). This result could be attribute to isoniazid was the most commonly used first line drug for *M. tuberculosis*. The frequency of two-drug resistant strains changed in different years, but was mainly resistant to SI (S+I). The summarized 2-drug resistant frequency was: SI>IR>SR>IE>SE>RE. The summarized 3-drug resistant frequency were: SIR (S+I +R)>SIE>IRE>SRE, and was mainly resistant to SIR (Table 1). The 4 drug resisted strains increased slightly from 2010 but was descending in general (Figure 1).

Monodrug resistance, polydrug resistance and multidrug resistance changing trend: The multidrug resistance rate increased steadily since 2008 overall. Monodrug resistant rate present wave shape trend, monodrug resistant rate increased significantly from 2010 to 2012 but declined in 2015. The polydrug resistance rate declined steadily increased significantly from 2011 to 2012 and changed slightly in recent 3 years (Table 2).

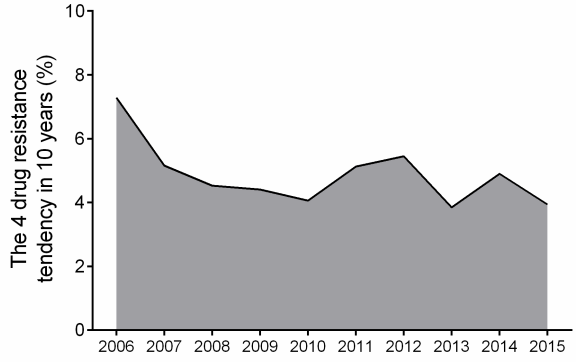

Figure 1. *The 4-drug resistance tendency from 2006 to 2015 (%).*

Table 1. *Resistance frequency of 4 first-line anti-tuberculosis drugs in different years.*

	Resistant drug	S	I	R	E	S+I	S+R	S+E	I+R	I+E	R+E	S+I+R	S+I+E	S+R +E	I+R+E	S+I+R+E
2006 (n=384)	Strain number	22	28	2	8	22	1	0	10	4	0	25	5	0	2	28
	Resistant rate (%)	5.73	7.29	0.52	2.08	5.73	0.26	--	2.6	4.17	--	6.51	1.3	--	0.52	7.29
2007 (n=504)	Strain number	32	41	4	2	44	1	0	9	5	1	21	3	0	4	26
	Resistant rate (%)	6.35	8.13	0.79	0.4	8.73	0.2	--	1.79	1	0.2	4.17	0.6	--	0.79	5.16
2008 (n=707)	Strain number	25	43	3	5	47	3	1	9	3	0	26	1	1	5	32
	Resistant rate (%)	3.54	6.08	0.42	0.7	6.65	0.42	0.14	1.27	0.42	--	3.68	0.14	0.14	0.7	4.53
2009 (n=816)	Strain number	39	73	10	8	52	6	0	16	0	0	34	3	0	8	36

	Resistant rate (%)	4.78	8.95	1.23	0.98	6.37	0.74	--	1.96	--	--	4.17	0.37	--	0.98	4.41
2010 (n=1011)	Strain number	29	74	6	--	60	4	0	18	0	1	62	6	0	2	41
	Resistant rate (%)	2.87	7.32	0.59	--	5.93	0.4	--	1.78	--	0.1	6.13	0.59	--	0.2	4.06
2011 (n=1305)	Strain number	40	87	23	9	74	2	0	26	6	1	69	13	1	13	67
	Resistant rate (%)	3.07	6.67	1.76	0.69	5.67	0.15	--	1.99	0.46	0.08	5.29	1	0.08	1	5.13
2012 (n=1393)	Strain number	84	96	12	6	111	5	3	35	2	0	72	11	1	4	76
	Resistant rate (%)	6.03	6.89	0.86	0.43	7.97	0.36	0.22	2.51	0.14	--	5.17	0.79	0.07	0.27	5.45
2013 (n=1479)	Strain number	69	110	17	7	97	1	1	24	7	0	77	8	0	7	57
	Resistant rate (%)	4.67	7.44	1.15	0.47	6.56	0.07	0.07	1.62	0.47	--	5.21	0.54	--	0.47	3.85
2014 (n=1631)	Strain number	83	130	8	19	109	1	9	39	16	0	99	18	0	17	80
	Resistant rate (%)	5.09	7.97	0.49	1.16	6.68	0.06	0.55	2.39	0.98	--	6.07	1.1	--	1.04	4.9
2015 (n=1626)	Strain number	89	96	8	4	109	2	0	30	3	0	92	9	1	11	64
	Resistant rate (%)	5.47	5.9	0.49	0.25	6.7	0.12	--	1.85	0.18	--	5.66	0.55	0.06	0.68	3.94

S: Streptomycin; H: Isoniazid; R: Rifampin; E: Ethambutol.

Table 2. *Single drug resistance, polydrug resistance, multi-drug resistance change trend in different years.*

	Monodrug		**Polydrug**		**Multidrug**	
Year	**Strain number**	**Resistant rate (%)**	**Strain number**	**Resistant rate (%)**	**Strain number**	**Resistant rate (%)**
2006	60/384	15.62	32/384	8.33	65/384	16.93
2007	79/504	15.67	54/504	10.71	60/504	11.9
2008	77/707	10.89	55/707	7.78	72/707	10.18
2009	130/816	15.93	61/816	7.48	94/816	11.52
2010	109/1011	10.78	71/1011	7.02	123/1011	12.17
2011	159/1305	12.18	97/1305	7.43	175/1305	13.41
2012	198/1393	14.21	130/1393	9.33	190/1393	13.64
2013	203/1479	13.73	106/1479	7.17	165/1479	11.16
2014	240/1631	14.71	135/1631	8.28	234/1631	14.35
2015	197/1626	12.12	114/1626	7.01	197/1626	12.12
Total	1452/10856	13.38	855/10856	7.88	1375/10856	12.68

Monodrug resistance: The *Mycobacterium tuberculosis* is confirmed to be resistant to 1 first-line anti TB drug; Polydrug resistance: resistant to more than 1 first-line anti TB drug but not include isoniazid and rifampicin simultaneously; Multidrug resistance: at least resistance to isoniazid and rifampicin simultaneously.

Discussions

Drug resistant to *M. tuberculosis* has become a major cause of tuberculosis spreading, and causes hard controlling in tuberculosis. The multi-drug resistant TB (MDR-TB) could cause poor cure rate and high cost of tuberculosis. MDR-TB serious impact on the work of TB control [15,16]. The total drug resistant rate was 33.92% in our study, the monodrug resistant order was isoniazid>streptomycin>rifampicin>ethambutol. As one of the main first-line anti-tuberculosis drugs, Isoniazid (I) has highly selective anti-bacteria activity against *M. tuberculosis*. Isoniazid mainly exerts effect on growing TB, and only has bacteriostatic effect on inactive TB. Isoniazid could penetrate

phagocytic cells easily, and has bactericidal effect on TB both inside and outside of the cell [17]. Isoniazid was used as the main chemotherapy drugs in clinical, it's drug resistant rate increased with the high used frequency. As one of first line tuberculosis drug, ethambutol has good clinical effect [18]. This study also showed that the single and multi-drug resistant rates to ethambutol were low, which indicating that it has a good application potential.

We found the main 2-resistant drugs were streptomycin (S) + isoniazid (I), and 3- resistant drugs were S+I+R (rifampicin). Isoniazid and Rifampicin are mainly combined used, the drug resistant rate to these 2 drugs increased year by year. Research indicated nearly 1/3 of the tuberculosis patients are resistant to Isoniazid and Rifampicin [19], which may cause by the excessive abuse of short course chemotherapy composed by Isoniazid and Rifampicin. Correctly using of Isoniazid and Rifampicin, thus reducing the drug resistant rate of *M. tuberculosis* is the main direction of our future work.

Our research also showed the multidrug resistance (MDR-TB) rate is 12.68% in 10 years. The most noteworthy is that the total multi-drug resistance rate showed a slightly increasing trend since 2008. The total multi drug resistant rate was much higher than the national (China) tuberculosis epidemiological survey statistics data (6.8%) and also higher than the Hu' reported MDR-TB rate [20]. This may attribute to the non-standard medication, repeated hospitalization of investigated patients. Because they have serious illness, or the most patients received multiple treatment. The increasing of MDR-TB patients has attracted worldwide attention. Cheepsattayakorn [21] indicated the host genetic factors are the cause of the occurrence, development and drug resistance of tuberculosis. We believe the rapid screening and gene polymorphism studies of isoniazid, rifampin resistance gene using advanced technology also play important role in screening drug resistance tuberculosis [22-24].

MDR-TB is the spreading foundation of tuberculosis. Ineffectively controlling of MDR-TB lead to more chronic infection and pan resistant bacteria, thus resulting in the difficulty in the furtherly treatment and management of tuberculosis increased. At the same time, resistant *M. tuberculosis* in the initial treated patients further increased the difficulty of treatment [25]. The fifth epidemiological survey of China estimated there are 900,000 new tuberculosis patients diagnosed each year, and about 50,000 patients are resistant to multi drug. However, there were only 2882 patient registered resistant to multi drug in 2012, and 1969 (68%) of them implemented treatment. This means less than 1% of TB patients registered. Strengthen the management and supervision of patients with drug resistant *M. tuberculosis* is the basis of controlling drug-resistant tuberculosis.

The emergence of resistant *M. tuberculosis* is caused by many reasons: (1) irregular medication is the main occurrence reason of drug-resistant *M. tuberculosis*. Chen et al. [26] indicated non-standard or irregular therapy is important risk factors for multidrug resistance in TB patients. (2) Un-reasonable chemotherapy, without using effective chemotherapy [27]. In recent years, WHO has provided a series of guiding principles for management of TB, which has important meaning for the treatment of tuberculosis. (3) mismanagement of chemotherapy, especially failed to implement supervising and managing TB in strengthening period [28]; (4) poor patient's compliance, patient did not take the medicine on time and complete the treatment; (5) patients did not able to get needed anti-TB drugs due to economic reasons, or the get poor quality of anti-TB drugs; (6) Patients admitted to tuberculosis hospitals are mostly have severe refractory tuberculosis, this is the important reasons for the high nosocomial resistance rate in our study. Therefore, it is very necessary to supervise tuberculosis patients strictly and control the source of infection in hospital. In addition, managing MDR-TB patients both by means of hospital management and non-hospital treatment. For patient diagnosed with MDR-TB or patient with adverse drug reactions, the hospitalization, implementation of clinical intervention, performing sufficient treatment to these patients could effectively curb the spread of drug-resistant *M. tuberculosis*.

If the patient with multi drug resistant who cannot be admitted to tuberculosis hospital, they should be reported to corresponding tuberculosis prevention and control unit and been supervised. Management of all types of drug resistant TB patients to improve the cure rate, reducing their recurrence rate, and reducing the mortality rate of drug-resistant TB could effectively prevent the propagation of drug resistant TB. On the other hand, strengthening the training of prevention and treatment of tuberculosis in medical staff, improving the alertness and early diagnosis of pulmonary tuberculosis patients; and early treatment could reduce the drug resistant *M. tuberculosis*, which could benefit the controlling of TB in the future.

In conclusion, our study showed that the resistant rate of *M. tuberculosis* to isoniazid was and resistant rate to ethambutol was low in local region, while the multidrug resistance (MDR-TB) rate was higher. This demonstrate that adjust the treatment plan of tuberculosis, strengthen the supervision and management are very necessary to control tuberculosis.

References

1. Gao L, Lu W, Bai L, Wang X, Xu J, Catanzaro A, Cardenas V, Li X, Yang Y, Du J, Sui H, Xia Y, Li M, Feng B, Li Z, Xin H, Zhao R, Liu J, Pan S, Shen F, He J, Yang S, Si H, Wang Y, Xu Z, Tan Y, Chen T, Xu W, Peng H, Wang Z, Zhu T, Zhou F, Liu H, Zhao Y, Cheng S, Jin Q. Latent tuberculosis infection in rural China: baseline results of a population-based, multicentre, prospective cohort study. Lancet Infect Dis 2015; 15: 310-319.
2. Cakir E, Erdem E, Ozlu N, Seber E, Gencer S, Kilicaslan Z. Demographic and microbial characteristics and drug resistance of childhood tuberculosis in Istanbul: analysis of 1,541 cases. J Infect Dev Ctries 2014; 8: 304-309.
3. Hu Y, Mathema B, Zhao Q, Zheng X, Li D, Jiang W, Wang W, Xu B. Comparison of the socio-demographic and clinical features of pulmonary TB patients infected with

sub-lineages within the W-Beijing and non-Beijing Mycobacterium tuberculosis. Tuberculosis (Edinb) 2016; 97: 18-25.

4. Eurosurveillance Editorial Team. WHO publishes Global tuberculosis report 2013. Euro Surveill 2013; 18.
5. Tuberculosis global facts 2010/2011. Cent Eur J Public Health 2010; 18: 197.
6. Technical Guidance Group of the Fifth National TB Epidemiological Survey TOotFNTES. The fifth national tuberculosis epidemiological survey in 2010. Chinese J Antitubercul 2012; 34: 485-508.
7. Chen Y, Zhao B, Liu HC, Sun Q, Zhao XQ, Liu ZG, Wan KL, Zhao LL. Prevalence of mutations conferring resistance among multi- and extensively drug-resistant Mycobacterium tuberculosis isolates in China. J Antibiot (Tokyo) 2016; 69: 149-152.
8. Qi YC, Ma MJ, Li DJ, Chen MJ, Lu QB, Li XJ, Li JL, Liu W, Cao WC. Multidrug-resistant and extensively drug-resistant tuberculosis in multi-ethnic region, Xinjiang Uygur Autonomous Region, China. PLoS One 2012; 7: 32103.
9. Chen J, Qi L, Xia Z, Shen M, Shen X. Which urban migrants default from tuberculosis treatment in Shanghai, China. PLoS One 2013; 8: 81351.
10. Chen M, Kwaku AB, Chen Y, Huang X, Tan H. Gender and regional disparities of tuberculosis in Hunan, China. Int J Equity Health 2014; 13: 32.
11. Liu M, Jiang W, Liu Y, Zhang Y, Wei X, Wang W. Increased genetic diversity of the Mycobacterium tuberculosis W-Beijing genotype that predominates in eastern China. Infect Genet Evol 2014; 22: 23-29.
12. Varghese B, Al-Omari R, Al-Hajoj S. Inconsistencies in drug susceptibility testing of Mycobacterium tuberculosis: Current riddles and recommendations. Int J Mycobacteriol 2013; 2: 14-17.
13. Zignol M, van Gemert W, Falzon D, Sismanidis C, Glaziou P. Surveillance of anti-tuberculosis drug resistance in the world: an updated analysis, 2007-2010. Bull World Health Organ 2012; 90: 111-119.
14. Simons SO, van Soolingen D. Drug susceptibility testing for optimizing tuberculosis treatment. Curr Pharm Des 2011; 17: 2863-2874.
15. Mitnick CD, Rodriguez CA, Hatton ML, Brigden G, Cobelens F, Grobusch MP, Horsburgh R, Lange C, Lienhardt C, Oren E, Podewils LJ, Seaworth B, van den Hof S, Daley CL, Gebhard AC, Wares F, Resist TB. Programmatic management of drug-resistant tuberculosis: an updated research agenda. PLoS One 2016; 11: 0155968.
16. Micheletti VC, Kritski AL, Braga JU. Clinical features and treatment outcomes of patients with drug-resistant and drug-sensitive tuberculosis: a historical cohort study in Porto Alegre, Brazil. PLoS One 2016; 11: 0160109.
17. Ge F, Zeng F, Liu S, Guo N, Ye H, Song Y, Fan J, Wu X, Wang X, Deng X, Jin Q, Yu L. In vitro synergistic interactions of oleanolic acid in combination with isoniazid, rifampicin or ethambutol against Mycobacterium tuberculosis. J Med Microbiol 2010; 59: 567-572.
18. Zhang Z, Yan J, Xu K, Ji Z, Li L. Tetrandrine reverses drug resistance in isoniazid and ethambutol dual drug-resistant Mycobacterium tuberculosis clinical isolates. BMC Infect Dis 2015; 15: 153.
19. The Lancet Infectious Diseases. The worldwide epidemic of multidrug-resistant tuberculosis. Lancet Infect Dis 2011; 11: 333.
20. Hu Y, Hoffner S, Wu L, Zhao Q, Jiang W, Xu B. Prevalence and genetic characterization of second-line drug-resistant and extensively drug-resistant Mycobacterium tuberculosis in Rural China. Antimicrob Agents Chemother 2013; 57: 3857-3863.
21. Cheepsattayakorn A, Cheepsattayakorn R. Human genetic influence on susceptibility of tuberculosis: from infection to disease. J Med Assoc Thai 2009; 92: 136-141.
22. Staff PO. Correction: A meta-analysis of P2X7 gene-762T/C polymorphism and pulmonary tuberculosis susceptibility. PLoS One 2015; 10: e0121649.
23. Chen M, Liang Y, Li W, Wang M, Hu L, Abuaku BK, Huang X, Tan H, Wen SW. Impact of MBL and MASP-2 gene polymorphism and its interaction on susceptibility to tuberculosis. BMC Infect Dis 2015; 15: 151.
24. Sun YP, Cai QS. Vitamin D receptor FokI gene polymorphism and tuberculosis susceptibility: a meta-analysis. Genet Mol Res 2015; 14: 6156-6163.
25. Zhou qing FZ, Fang XM, XU LY. Variance of antimicrobial resistance of mycobacterium tuberculosis in clinical treatment. Chin J Nosocomiol 2009; 19: 34-37.
26. Chen S, Huai P, Wang X, Zhong J, Wang X, Wang K, Wang L, Jiang S, Li J, Peng Y, Ma W. Risk factors for multidrug resistance among previously treated patients with tuberculosis in eastern China: a case-control study. Int J Infect Dis 2013; 17: 1116-1120.
27. Zhang W, Wu Q, Lin Y. Analysis of reasons failed focal debridement in 109 patients with spinal tuberculosis. Zhonghua Jie He He Hu Xi Za Zhi 1998; 21: 181-183.
28. Moore DA. What can we offer to 3 million MDRTB household contacts in 2016? BMC Med 2016; 14: 64.

*Correspondence to

Wu Qi

Department of Clinical Laboratory

Tianjin Haihe hospital

PR China