Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature: _____ Date: _____

Drug Susceptibility of *Mycobacterium tuberculosis* from 10 Provinces in China, 2004 – 2006

By

Yang Zhang

MPH

Epidemiology Department

Dr. Scott McNabb

Committee Chair

Drug Susceptibility of *Mycobacterium tuberculosis* from 10 Provinces in China, 2004 – 2006

By

Yang Zhang

M.P.H, Emory University 2013

Thesis Committee Chair: Dr. Scott McNabb

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health in Global Epidemiology
2013

Abstract

Background: The prevalence of drug-resistant tuberculosis (DRTB) threatens TB control. Little is know about this in China. We conducted a drug-resistance survey in 10 provinces of China among TB patients from 2004 – 2006 to understand the distribution of DRTB and make policy recommendations.

Methods: The proportions of DRTB were determined from provincial surveys that included all clinical *Myobacterium tuberculosis* strains (Mtb) tested for susceptibility to isoniazid, rifampin, ethambutol, and streptomycin by means of the absolute concentration and the proportional drug susceptibility testing methods.

Results: Among DRTB the greatest percentage were single drug-resistant (SDR) TB (29.1%). Sichuan had the largest percentage of multiple drug-resistance (MDR) TB (38.7%). For SDR TB, being female (OR=1.18; 95%CI=0.88-1.57), having been previously treated (OR=1.48; 95%CI=1.08-2.04), and being < 15 years old were risk factors. For MDR TB, being male (OR 1.12; 95%CI=0.86-1.46), having been previously treated (OR 5.46; 95% CI=4.12-7.24), and being < 15 years old were risk factors.

Conclusions: China has a serious epidemic of DRTB, especially MDR TB, and the status varied between provinces. Patients with previous treatment history were at higher risk for DRTB. China was much higher compared to the global estimated average of 4.8%.

Drug Susceptibility of *Mycobacterium tuberculosis* from 10 Provinces in China, 2004 – 2006

By

Yang Zhang

MPH

Epidemiology Department

Dr. Scott McNabb

Committee Chair

ACKNOWLEDGEMENTS

Thank you to Dr. Scott McNabb, for agreeing to be my committee chair and for patiently fostering me through this process.

Thank you to Dr. KangLin Wan for all his help when I was working on this project in China.

Thank you to numerous public health personnel in Tuberculosis Department in Chinese Center for Disease Control and Prevention who assisted with this work.

CONTENTS

INTRODUCTION

Tuberculosis (TB) remains the most significant infectious diseases threatening global safety (1, 2). In the light of the report of World Health Organization (WHO), China has the second-highest incidence of TB and the third-highest incidence of multiple drug-resistant of TB (MDR TB) worldwide; registering 17% of the global TB cases in 2010.(3) "MDR TB is defined as TB caused by strains of *Mycobacterium tuberculosis* (Mtb) resistant to at least isoniazid (INH) and rifampicin (RFP)."(4) Prevalence of TB has decreased in China over the last decades, however, the drug-resistant (DR) rates of TB have increased, especially MDR, which raises concern. (5, 6) Compared to patients with susceptible TB, the MDR TB treatment program is less effective.(7) The proportion of DRTB is a useful indicator when assessing the performance of a TB control program. (8) Based on the data of the 5th Nationwide Random Survey for the Epidemiology of TB in China in 2010, it estimates that there are 5 million TB cases in China.

The single DRTB proportion is 36.8% (95%CI=31.1%-42.7%) and the MDR TB proportion is 6.8% (95%CI=4.1%-10.4%). (9) However, the sample size of the isolates to test DR was only 280, which is too small to be capable to stratify the data according to province. Estimate of DRTB in China relies on local hospitals or regional CDC surveys. (3) We can just find the results of the DR surveys conducted in several individual provinces in a specific period or cohort studies in one hospital in China in the international scientific literature. (ref) However, the statistics of the magnitude and pattern of DRTB in China always shows inconsistently in different regions on account of diversity of ethnic and population density, heterogeneous customs, geographically distributed and the size of the province, the unbalanced social-economic and other factors (3, 10).

Previous studies shows that more than 52 factors are related to DR, in the meantime identifies several risk factors. (5) However, besides previous treatment history, sex and HIV infection, the risk factors reported by different study populations are heterogeneous (8). Some study reports that being male is strongly associated with DRTB, but the association is weak in other studies; some study reports that non-permanent residents, migration and younger are predictors of MDR TB, but results are insignificant in other studies.(5, 8, 11-13) Most risk factors for drug-resistant of TB in China are still largely unknown.

This study surveyed the mainland of China from east to west, covering 36% (465 million out of 1.3 billion inhabitants) of the total Chinese population and tested the drug susceptibility of Mtb from 10 of 31 provinces in China from 2004 – 2006. This contributes to a better understanding of the regional epidemiology of MDR TB. It reports the proportion of DRTB and aims to identify the risk factors related to MDR TB to help China improve TB case management.

REVIEW OF LITERATURE

Definitions: (1)

"Drug resistance rate is the rate strains become resistant as assessed by drug susceptibility tests (e.g., the number of drug resistance strains/ the number of examined strains * 100%) including single drug resistance (SDR) rate and multiple drug resistance (MDR) rates."

MDR TB usually means Mtb has resistance against more than two first-line anti-Mtb drugs. According to the guideline of DR surveillance published by WHO, "MDR TB is defined as the resistance against ≥ 2 anti-Mtb drugs at least including INH and RFP."

"New cases: TB patients who denied having prior anti-TB treatment or who received anti-TB treatment for 30 days or less."

"Previously treated case: a newly registered episode of TB in a patient who, in response to direct questioning admits having been treated for TB for one month or more, or, in countries where adequate documentation is available, there is evidence of such history."

"Relapsed case: A patient previously treated for TB and declared cured by a medical officer after one full course of chemotherapy, but who reports back to the health service bacteriologically positive (smear or culture)."

"Primary Drug Resistance (PDR): PDR concerns Mtb isolated from patient which never used any anti-Mtb drug or used anti-Mtb drug for < one month."

"Acquired drug resistance (ADR): After treatment has started, drug-resistant variants may emerge

although the bacterium initially isolated from the patient was sensitive to drugs."

Introduction of TB

TB is a communicable bacterial disease caused by *Mycobacterium tuberculosis* (Mtb), that in most cases affects the lungs, with transmission through the active respiratory disease from person to person. (6) The symptoms of pulmonary TB are cough, always with phlegm and sometimes with haemoptysis, fever, chest pain, breathlessness, low appetite, wizened and sweats.(9) The course of treatment is usually six months. (14) The highest burden of TB is in Asia and Africa. The sum of Indian and China TB patients accounted for nearly 40% of the world's TB cases. On a global scale, about 4% of new cases and 20% of previously treated cases were estimated to be with MDR TB. (15) It was untoward to estimate the burden of TB in children who were less than 15 years old so WHO excluded it except the first report. The reported of global TB in 2012 estimated that there were 0.5 million cases among children in 2011. (15) So in our study, we selected Mtb isolates through sputum smear and distinguished those to six age groups beginning with < 15.

TB infection and Drug-resistance

As Crofton stated, "the greatest disaster that can happen to a patient with TB is that his organisms become resistant to two or more of the standard drugs."(3) Drug resistance was often in order to the transmission of infrequently resistance clones with generated through mutation or acquisition of resistant gene-bearing mobile genetic elements.(15) It was always insufficient for a most common bacterial infection to generate of resistant because their short treatment course (less than two week). However, treatment of TB of lung infection definitely required > six months to use the

four first-line drugs, which gave Mtb abundant time and opportunity to develop drug resistance.(2) According to the WHO, among 107 countries reported treatment outcomes, only 30 of them achieved the target treatment success rate (=>75%) for patients with MDR TB. (15) Relapse case, previous treated case, and other kings of case were provided severe definitions. When analyzed the previous treated history, we pay attention to the outcomes and period of the former treatment. Moreover, we should focus on the drug use history and measures of therapy of patients if possible. The different patterns of drug resistance could also give us some clue to find the risk factors of MDR TB.

China was the second highest incidence of TB according to the WHO. Based on the data of the 4th Nationwide Random Survey for the Epidemiology of TB in China in 2000, there were 2 million bacteriologically confirmed pulmonary TB patients among of 4.5 million prevalent TB patients in China. In addition, the data of the 5th Nationwide Random Survey for the Epidemiology of TB in China in 2010 made a conclusion that there were 5.0 million prevalent TB cases in China, which reflected a tendency of increase.(9) For the sake of gaining insight into the prevalence and distribution of DRTB and enhance the controlling ability, China joined the anti-TB drug-resistant surveillance project all over the world by WHO. The first nationwide DR survey for this project was conducted in 2007, estimated 22% of global burden MDR-TB.

We conclude that the consequences for DR were consistent with values estimated by WHO formerly from subnational studies. However, we could not find the details about the survey design and specific data in each province in China from published articles. From the survey, we can just know that the total proportion of MDR-TB was 8.32% (95% CI=7.13–9.74), with the proportion of

MDR-TB in new cases 5.75% (95% CI=4.62–7.17) and in previously treated cases 25.67% (95% CI=21.72–30.01). However, we noticed that the single resistant of TB was 21.33% and MDR-TB was 8.25% among new cases while the single resistant of TB was 20.03% and MDR-TB was 9.56% among previously treated cases based on the National Baseline Survey of DRTB. There were a definitely diversity between the results of MDR-TB in previous treated cases. To find a reasonable explanation of that variant, I read the literature. A meta-analyses research, which included huge sample size, published in 2011 showed that the prevalence of MDR-TB in new cases was 5.3% (95% CI=4.4%–6.4%) and in previously treated cases was 27.4% (95% CI=24.1%–30.9%) respectively, which are similar with the results obtained by nationwide survey in 2007. Compared those surveys, we found that the national baseline was just included small sample size and several provinces, which may not represented the situation in China. The results found to be frequently heterogeneous in many published articles revealed that geographic distribution, population density and mobility, quality of drug susceptibility testing, etc. were related to the results of MDR-TB.

Method of testing drug susceptibility of Mtb

Here are 3 sampling strategies for monitoring DR:(3)

- Randomly selected clusters of patients
- Selected of all diagnostic centers during a specified period
- Cluster sampling. (Sampling of all diagnostic centers and selected the proportional to the number of cases during a specified period)

Bacteriologic Test: (16)

To test the biochemical identification of the bacterial strains, we always use P-nitro-alpha-acetylamino-beta-hydroxypropiopheneone (NAP)， 2-thiophene-2-carboxylic acid hydrazide (TCH) and Löwenstein–Jensen (L-J) tests.

Species of *Mycobacterium*	NAP	TCH	L-J
M.tuberculosis	-	+	+
M.bovis	-	-	+
non-*M.tuberculosis*	+	+	+

Means of absolute proportion method was always used to test drug susceptibility.

Here are the concentrations for the four tuberculosis drugs in the survey:

Isoniazid (INH):0.2 μg /ml

Rifampicin（RFP）: 40 μg/ml

Streptomycin (SM) : 4 μg/ml

Ethambutol (EMB) : 2 μg/ml.

The critical growth proportion for resistance was defined as 1% for all drugs.

DRTB in China

There were a small number of papers that reported the results of the DR surveys conducted in China in international scientific journals. (17-19) Among this small quantity of articles, some conducted retrospective studies to analyze the trend of the proportion in specific individual provinces or referral hospitals; (20-26) some used meta-analyses included published articles to make the overview of prevalence of DR in China;(27) some national surveys published overall

analysis of DRTB included several provinces in China,(8) however, they didn't compare the difference between provinces and the period of survey was also limited. According to many articles, the statistics of the magnitude and pattern of DRTB in China always showed inconsistently in different regions on account of diversity of ethnic and population density, heterogeneous customs, geographically distributed and the size of the province, the unbalanced social-economic and other factors (3, 10).

Risk Factors of DRTB

TB affects the low and middle income countries according to WHO.(6) So the lower economic was considered to be a risk factor. Moreover, some articles concluded that development of DR was strongly connected with social factors, such as unemployment and alcohol drinking, that may also related to the social-economic status.(12) The availability of disseminate MDR-TB also depended on the prevalence of DSTB and the mobility of patients.(13) Making patients immunized with protection by exposure to DSTB was an important method to prevent MDR-TB. However, the single population successfully declined with incidence of drug-susceptible of TB could reduce whole population immunity to of MDR-TB and enhance the probability and vulnerability of an epidemic. (28) From the above, it indicated a uncommon sense that countries with good TB control might be more vulnerable to MDR-TB.(28) In contrast, small scales of poor control in promotion of case detection and Directly-Observed Treatment Strategy (DOTS) might control their risk of MDR-TB.(28) It might give us a direction to control MTB-TB in herd population rather than small scale managing. In addition, many literatures showed that inadequate and unreasonable treatment of TB was strongly associated with MDR-TB. DRTB

varied apparently across the outcome of different previous treatment. (17) Furthermore, a literature concluded that previously treated history, lower-education status, without health insurance were risk factors of MDR-TB also.(29) In conclusion, we should focus on the circumstance of inadequate and unreasonable treatment of TB, which were also related to those above, when analyzed the risk factors. Besides, prevention of reinfection also should be considered. Previous studies showed that more than 52 factors were related to drug- resistant in the meantime identified several risk factors.(5) However, besides previous treatment history, sex and HIV infection, the risk factors reported by different study populations are heterogeneous(8). Some studies reported that male sex is strongly associated with DRTB, but the association is weak in other studies; some studies reported that non-permanent residents, migration and younger were predictors of MDR-TB, but results were insignificant in other studies.(5, 8, 11-13) We need to test it use our own dataset.

METHODS

Data resources and sampling method

Patients were selected from TB hospitals and institutes for TB control and cure. This study enrolled all clinical Mtb strains from TB patients during a specific period. Jilin, Beijing, Zhejiang, Fujian, Henan, Hunan, Xinjiang, Gansu, Sichuan, Guangxi were survey areas chosen between 2004 and 2006, with the sample size of 349, 108, 97, 479, 98, 102, 186, 220, 106, 209, respectively. The geographical location of the provinces is in Figure1. Demographic data using a standardized questionnaire were collected and entered into a computer database using Microsoft Access software, including identification number, living situation, gender, age occupation and etc. We also supplemented the clinical information of treatment record of TB.(1)

Mtb Selection and Drug Susceptibility Testing (DST)

A total of 1958 clinical strains from TB patients in 10 provinces were tested for susceptibility to four first-line, anti-TB drugs included INH, SRM, RFP and EMB. Culture was performed by the Tuberculosis Reference Laboratory (TRL) in Beijing and kept in the National Laboratory of TB, SKLID/ICDC, China CDC, and Research Institute for Tuberculosis Control.

To test the biochemical identification of the bacterial strains, we used P-nitro-alpha-acetylamino-beta-hydroxypropiopheneone (NAP)， 2-thiophene-2-carboxylic acid hydrazide (TCH), and Löwenstein–Jensen (L-J) media to distinguish Mtb. (3)

Species of *Mycobacterium*	NAP	TCH	L-J
M. tuberculosis	-	+	+

M. bovis	-	-	+
non *M. tuberculosis*	+	+	+

The absolute concentration and proportional drug susceptibility testing methods were used to test drug susceptibility (3).

These are the concentrations for the four TB drugs under survey:

Isoniazid (INH):0.2 μg /ml

Rifampicin（RFP: 40 μg/ml

Streptomycin (SM): 4 μg/ml

Ethambutol (EMB): 2 μg/ml.

The critical growth proportion for resistance was defined as 1% for all drugs.

Statistical Analyses

Methods published by WHO were used to calculate the proportion of DRTB in China. (3) We estimated the proportion of DRTB in 10 provinces from east to west in China. Patients were considered new cases of TB if they denied a history of having any prior anti-TB treatment or having received anti-TB treatment for less or equal to 30 days. (3) Patients were considered to be previously treated if they have been treated for one month or more. (1) We compared the proportion of single drug-resistant (SDR) and MDR-TB by age, gender, and previous treatment history of TB. Except distinguished age group to 6 parts began with < 15 years of age to > 55 years of age; others were bivariate variables. Pearson Chi-square test was used to comparison the categorical variables by different groups. Odds ratios (ORs) and 95% confidence intervals

(CI) were calculated to measure the relationship between characteristics of patients and the DRTB. (12) A p-value <0.05 was considered statistically significant. All statistical analyses were done using SAS 9.3. Data were checked for missing values and for all variables. Each variable was analyzed individually to ascertain if there were implausible values.

RESULTS

General

Patient recruitment began 2004 and ended 2006. However, 10 provinces had different periods of survey enrolled (Table 1). Mtb strains isolated from 1958 patients were analyzed by DST. Four patients did not have Mtb. For the rest of the 1954 patients, the average age was 47.5 years old, included 1297 males and 657 females. Many, 1388 (71.5%) were treated for the first time, and 556 (28.5%) had been treated for TB for one month or more. The locations and relative area for 10 Chinese provinces are shown in Figure 1.

Single Drug TB Resistance

Among 1954 *Mtb* isolates, the total proportion of first-line single TB drug resistance (SDR) in 10 provinces was 11.2%. INH had the highest rate of resistance (21.6%), followed by SM (19.9%), RFP (19.2%), and EMB (11.4%) (Table 2). There was a large diversity of SDR in the interior of the east, the west, and the middle. Gansu had the largest percentage of SDR (29.1%). The sequential distributions (from high to low) were Zhejiang (23.7%), Xinjiang (17.2%), Guangxi (12.9%), Henan (10.2%), Beijing (10.2%), Hunan (8.8%), Fujian (7.3%), Sichuan (3.8%), and Jilin (1.2%). The separate proportions of four first-line TB drug resistance were dissimilar in 10 provinces. The highest rate of SDRTB in 10 provinces was the resistance of SM in Gansu (19.6%) (Table 3), followed by EMB in Zhejiang (15.5%) (Figure 2).

MDR-TB

A total of 280 (14.3%) Mtb isolates were resistant to at least INH and RFP in 10 provinces. Among

MDR TB, 116 were identified from new patients; 164 from previously treated patients, with the rate of MDR TB of 8.4% and 29.5%, respectively. Resistance of first-line drugs had the highest rate (6.5%), followed by three drugs (including INH, RFP and SM) (3.7%). There was a large diversity of MDR TB in the interior of the east, the west, and the middle. Sichuan had the largest percentage of MDR TB (38.7%). The sequential distributions (from high to low) were Fujian (26.9%), Hunan (24.5%), Zhejiang (12.4%), Guangxi (11.5%), Henan (8.2%), Xinjiang (5.9%), Beijing (4.6%), Gansu (4.5%), Jilin (4.3%) (Table 3 and Figure 2). The separate proportions of four first-line drug resistance were dissimilar in ten provinces.

Multiple Drug-resistant TB

A total of 129 (6.5%) isolates were resistant to > one first-line drug except MDR TB in 10 provinces. Among the multiple drug-resistant isolates, 96 were identified from new TB cases and 31 were from previously treated patients, with the rate of MDR TB 6.6% and 6.8%, respectively. Resistance of INH and SM had the highest rates (2.5%), followed RFP and SM (1.7%) (Table 3 and Figure 2).

Analysis of Risk Factors for Drug-resistant TB

Table 4 shows results of bivariate analyses of risk factors for SDR and MDR TB, respectively. All p-values were < 0.5. Therefore, female (OR 1.18; 95%CI=0.88–1.57), previously treated (OR 1.48; 95%CI=1.08-2.04) and < 15 year olds were associated with increased risk of acquiring SDR. Male (OR 1.12; 95%CI=0.86-1.46), previous treated (OR 5.46; 95% CI=4.12-7.24) and < 15 years old were associated with increased risk of acquiring MDR (Table 4).

DISCUSSION

The resistance to four, first-line, anti-TB drugs among pulmonary TB patients in the 10 provinces from 2004 to 2006 is higher than the average in the world, especially the emergence of MDR TB. (15) When compared to the reports before 2004, it shows a small decline in drug resistance, except some special provinces. (10)

In China, CDC attaches great importance to the dissemination of DR TB since its 4th Nationwide Random Survey for the Epidemiology of TB in China in 2000. (15) Monotherapy was found to be ineffective and contributed to drug resistance. (30) China joined the global program in 2007 to fight drug resistance, especially MDR TB. (3) Routine, first-line, anti-TB DST was not included in the standard care of TB patients according to the National Tuberculosis Program in China (because of prohibitive cost). (30) The 5th Nationwide Random Survey of Tuberculosis was conducted in 2010. It lacked sufficient data about TB drug resistance.

Therefore, our survey of TB drug susceptibility is a good step to determine the status of that problem as well as establishing continuous TB drug-resistant PHS. Our survey included the mainland of China from east to west. From the 4^{th} and 5^{th} National Epidemiological Survey of Tuberculosis in 2000 and 2010, we know that the TB prevalence in western area was higher than in the middle and eastern areas. (9) The corresponding lower clinical and public health levels and lower social-economic status may contribute to the phenomenon. (31, 32) However, our results don't show the same tendency. They show variability of drug resistance among the provinces. Our analysis indicated that the SDR in Gansu (29.1%) and Zhejiang (23.7%) and MDR in Sichuan (38.7%), Fujian (26.9%), and Hunan (24.5%) are much higher than the baseline

while Jilin had the lower SDR and MDR. We can find that MDR in the southwest is higher than other districts and northeast is lower than other districts. When examine the difference between these provinces, the variant of mobility and density of population may account for the unbalance.(33) Sichuan is one of the areas with highest population density among all provinces in China. (34) Besides, Sichuan and Gansu are the highest in China.

Zhejiang and Fujian absorbs a large number of immigrants each year; this can enhance the spread of TB. (21, 35-38) However, in this study, we do not have enough data to specifically examine migrating populations with respect to TB incidence. Moreover, the results in this study prove that RFP resistance may be a good marker for MDR in most case.(36)

When compared to the data before 2004, our data showed a slight decline in drug resistance except in some provinces (e.g., Sichuan, Fujian, and Hunan). (10) This may be for several reasons. First, there is an availability of anti-TB drugs without a prescription; second, there is poor DOTS implication, insufficiency of supervision of therapy, a lack of drug management, and inadequate of infection control measures in hospitals. (33, 39, 40) We need to monitor the therapy and drug treatment of TB. This can give us a more comprehensive understanding of drug resistance in overall China. (41-43)

Drug resistance is acquired for several ways, such as transmitted directly from infected drug-resistant TB patients, lack of management of treatment.(33) Spontaneous genetic mutations and acquisition amplified through selection reassure and further aggravated by poor adherence of patients gave rise to the DR TB.(44) Further, initial resistance and acquired resistance requires distinct control strategy so that we should realize the importance of

transmission patterns.(36) In the future, we should analyze patterns of transmission across different area.

As proved in many other studies, previous treated history has been consistently associated with the risk of MDR-TB. (45, 46) A review of MDR-TB with a focus on sub-Saharan Africa revealed a higher prevalence in previous treated cases than in new one. (11) Our finding (OR 5.46; 95% CI=4.12) is consistent with other results that illustrated the importance of previous treatment history in the risk of the development of drug resistance.

However, to gain a deeper understanding between the relationship of drug resistance and treatment history, further studies should be conducted with other groups (e.g. who relapse after successful treatment, who return with default, who have experience of failure previous treatment). (11, 45)

Based on the result of the 5th National Epidemiological Survey of Tuberculosis in 2010, the majority of TB patients are male. In our study, the number of males is also greater. However, the gender of TB patients may have different status of risk for drug resistance. The results may show that females develop SDR more readily than males, while males may be at increased risk of acquiring MDR (OR 1.12; 95%CI=0.86-1.46). In other studies, findings are inconsistent.(47-51) We don't have enough exposure data (e.g. the difference in access to health care). We need to do more analyses to discover gender disparities linked with drug resistance.

Diverse cut-off points are used when estimated association between age and drug-resistant TB. Their relationship is not well established, however, it always included <15 group. (3) In this study, we refer the national report of *Mtb* to distinguish the patients to 6 groups. (9) In our

study, the patients less than 15 has the highest risk of drug resistant, however, the total number of the children with drug resistant in our study is only 7, which may lead bias. Some analysis revealed the patients younger than 25 have the highest pooled risk of MDR.(52) Our statistic is consistent to the former results which illustrated that the frequency of MDR-TB is much higher in younger patients than older patients who are less than 44. Patients whose age is higher than 45 and less than 54 have the lowest risk of MDR-TB. The risk is increased after 55 years old. It may due to their body function or the diversity in life-style.(33)

Limitations

The method of data collection is not according to the sampling strategies for monitoring of drug resistance, which may lead bias and reduce effectiveness to the following analysis and cannot show the trend of drug-resistance. And we cannot analysis the prevalence of drug resistant based on this survey. In future, we should do the cluster sampling, proportional to the number of cases notified by the sampling of all diagnostic centers during a specified period. (3) However, the sample size per province in this dataset is much higher than other studies in the same period. In all, pay attention to the population with a large area of expanded geography can help us understand the transmissions of drug resistant clearly, especially MDR-TB.

The misclassifications are still unavoidable, in spite of interviewing patients with questionnaire and review of the medical records carefully to check previous therapy of TB, which are easier to make a potential bias when estimated drug resistant stratified by previous history. (53) Moreover, the further study should not be examined rather than merely previous treated group, also the group who relapse after successful treatment, who return with default, who relapse with

experience of failure previous treatment and etc. In future, we could train the data collector and interview patients face to face to get the accurate result.(53) In addition, the prevalence of XDR-TB is unclear because we do not perform DST for second-line anti-TB drugs in this study. Therefore, we should check them in further study.

In this study, the patients are lack of supervision of treatment. In China, it's difficult to do the routine DST of TB and follow-up interview.(46) We need to monitor the therapy and drug treatment of TB.

CONCLUSION

In conclusion, China has a serious epidemic of drug resistant TB, above-average of global estimated 4.8%, and the status varied obviously between provinces and.(33) High proportion of MDR-TB (14.30%) in ten provinces has become a main challenge for TB control. Patients with previous treated history have higher risk of drug resistant of TB, especially MDR-TB. Continuous surveillance of drug-resistance should be enhanced according the guideline by WHO in order to assess trends and help prevent and control it better.(3)

RECOMMENDATIONS

We recommend training for data collectors. It is necessary for all provinces in China to conduct their own continuous surveys and routine DST for TB to evaluation trends.(3) Provincial surveys should also consider for the different previous treatment outcome including successful therapy, treatment default or failure rather than merely previous treated.(11) It should also be tested the drug resistant of second-line drugs in spite of first-line drugs. Moreover, established the surveillance system included the drug treatment of TB and HIV.(2)

REFERENCES

1 WHO. Tuberculosis global report, 2008

http://www.who.int/tb/publications/2008/drs.report4_26feb08.pdf.

2 Goldberg DE, Siliciano RF, Jacobs WR, Jr. Outwitting Evolution: Fighting Drug-Resistant TB, Malaria, and HIV. Cell. 2012 Mar 16;148(6):1271-83.

3 Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response.

4 World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis Emergency update 2008. WHO/HTM/TB/2008.402. Geneva: World Health Organization,2008.

5 Chiang C-Y, Centis R, Migliori GB. Drug-resistant tuberculosis: Past, present, future. Respirology. 2010;15(3):413-32.

6 Akachi Y, Zumla A, Atun R. Investing in improved performance of national tuberculosis programs reduces the tuberculosis burden: analysis of 22 high-burden countries, 2002-2009. The Journal of infectious diseases. 2012 2012 May (Epub 2012 Mar;205 Suppl 2:S284-92.

7 Zumla A, Abubakar I, Raviglione M, et al. Drug-Resistant Tuberculosis-Current Dilemmas, Unanswered Questions, Challenges, and Priority Needs. Journal of Infectious Diseases. 2012 May 15;205:S228-S40.

8 Yanlin Z, Shaofa X, Lixia W, et al. National survey of drug-resistant tuberculosis in China.(Clinical report). The New England Journal of Medicine.366(23):2161.

9 The report of 5th Nationwide TB Prevalence Survey in China.

10 He GX, Zhao YL, Jiang GL, et al. Prevalence of tuberculosis drug resistance in 10 provinces of China. BMC infectious diseases. 2008;8:166.

11 Migliori GB, Dheda K, Centis R, et al. Review of multidrug-resistant and extensively drug-resistant TB: global perspectives with a focus on sub-Saharan Africa. Tropical Medicine & International Health. 2010 Sep;15(9):1052-66.

12 Balabanova Y, Radiulyte B, Davidaviciene E, et al. Risk factors for drug-resistant tuberculosis patients in Lithuania, 2002-2008. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology. 2012 2012-May;39(5):1266-9.

13 Liu CH, Yang N, Wang Q, et al. Risk factors associated with fluoroquinolone-resistant tuberculosis in a Beijing tuberculosis referral hospital. Respirology. 2011 Aug;16(6):918-25.

14 http://www.who.int/topics/tuberculosis/en/.

15 GLOBAL TUBERCULOSIS REPORT 2012

http://www.who.int/tb/publications/global_report/en/index.html.

16 Canetti G, Froman S, Grosset J, et al.

Mycobacteria: laboratory methods for test-

ing drug sensitivity and resistance. Bull

World Health Organ 1963;29:565-78.

17 Advances in research of extensively drug-resistant tuberculosis FAN Xiao-Ping, ZHAGN Wen Hong Department of Infectious Diseases, Hu ashan Hospital , Fudan Univer sit y, Shanghai 200040 , China.

18 Analysis on Drug-resistant S trains of 268 TB Patients w ith In itialTreatment in Sh ijiazhuang

City.HE L i-xin, ZHU Jiang-liang, ZHAO Zi-yun. Occup andHealth Vo.l 26 No. 1 Jan 2010.

19 Analysis of 451 anti-TB drug resistance surveillance in Fujian province. ZHAO Yong, LIN Jian, etal. CHINA TROPICAL MEDICINE Vol.11 No.10 October 2011.

20 Shen X, DeRiemer K, Yuan ZA, et al. Drug-resistant tuberculosis in Shanghai, China, 2000-2006: prevalence, trends and risk factors. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2009 Feb;13(2):253-9.

21 Zhao Y, Feng Q, Tang K, et al. The population structure of drug-resistant Mycobacterium tuberculosis clinical isolates from Sichuan in China. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2012 2012-Jun;12(4):718-24.

22 Wang D, Yang CE, Kuang TJ, et al. Prevalence of Multidrug and Extensively Drug-Resistant Tuberculosis in Beijing, China: a Hospital-Based Retrospective Study. Jpn J Infect Dis.63(5):368-71.

23 Wang J, Liu Y, Zhang C-L, et al. Genotypes and Characteristics of Clustering and Drug Susceptibility of Mycobacterium tuberculosis Isolates Collected in Heilongjiang Province, China. Journal of Clinical Microbiology. 2011 Apr;49(4):1354-62.

24 Wang X, Fu Q, Li Z, et al. Drug-resistant tuberculosis in Zhejiang Province, China, 1999-2008. Emerging infectious diseases. 2012 Mar;18(3):496-8.

25 Tang S, Zhang Q, Yu J, et al. Extensively drug-resistant tuberculosis at a tuberculosis specialist hospital in Shanghai, China: Clinical characteristics and treatment outcomes.

Scandinavian Journal of Infectious Diseases. 2011 Apr;43(4):280-5.

26 He GX, Xie YG, Wang LX, et al. Follow-up of patients with multidrug resistant tuberculosis four years after standardized first-line drug treatment. PloS one. 2010;5(5):e10799.

27 Yang Y, Li X, Zhou F, Jin Q, Gao L. Prevalence of Drug-Resistant Tuberculosis in Mainland China: Systematic Review and Meta-Analysis. PloS one. 2011 Jun 3;6(6).

28 Bishai JD, Bishai WR, Bishai DM. Heightened Vulnerability to MDR-TB Epidemics after Controlling Drug-Susceptible TB (MDR TB Epidemics). PloS one.5(9):e12843.

29 He GX, Wang HY, Borgdorff MW, et al. Multidrug-resistant tuberculosis, People's Republic of China, 2007-2009.(RESEARCH). Emerging infectious diseases.17(10):1831.

30 Shao Y, Yang D, Xu W, et al. Epidemiology of anti-tuberculosis drug resistance in a chinese population: current situation and challenges ahead. Bmc Public Health. 2011 Feb 17;11.

31 Long Q, Smith H, Zhang T, Tang S, Garner P. Patient medical costs for tuberculosis treatment and impact on adherence in China: a systematic review. Bmc Public Health. 2011 May 26;11.

32 Manjourides J, Lin H-H, Shin S, et al. Identifying multidrug resistant tuberculosis transmission hotspots using routinely collected data. Tuberculosis. 2012 May;92(3):273-9.

33 Wang W, Wang J, Zhao Q, et al. Contribution of rural-to-urban migration in the prevalence of drug resistant tuberculosis in China. European Journal of Clinical Microbiology & Infectious Diseases. 2011 Apr;30(4):581-6.

34 National bureau of statistics of the People's Republic of China. In 2010 tsncmdbnAahwsg.

35 Hu Y, Mathema B, Jiang W, Kreiswirth B, Wang W, Xu B. Transmission Pattern of Drug-Resistant Tuberculosis and Its Implication for Tuberculosis Control in Eastern Rural China.

PloS one. 2011 May 12;6(5).

36 Pang Y, Zhou Y, Zhao B, et al. Spoligotyping and Drug Resistance Analysis of <italic>Mycobacterium tuberculosis</italic> Strains from National Survey in China. PloS one. 2012;7(3):e32976.

37 Zhang L, Ye Y, Duo L, et al. Application of Genotype MTBDRplus in rapid detection of the Mycobacterium tuberculosis complex as well as its resistance to isoniazid and rifampin in a high volum laboratory in Southern China. Molecular Biology Reports. 2011 Mar;38(3):2185-92.

38 Qi Y-C, Ma M-J, Li D-J, et al. Multidrug-resistant and extensively drug-resistant tuberculosis in multi-ethnic region, Xinjiang Uygur Autonomous Region, China. PloS one. 2012 2012;7(2):e32103.

39 Shi D LH, Zhao Y, Jia Q, Coulter C, Li L, Zhu G. Extensively drug-resistant tuberculosis, Central China, 2007-2009. Emerging infectious diseases.2012 Nov;18(11):1904-5. doi: 10.3201/eid1811.120046.

40 Wan K, Liu J, Hauck Y, et al. Investigation on Mycobacterium tuberculosis Diversity in China and the Origin of the Beijing Clade. PloS one. 2011 Dec 29;6(12).

41 Liu CH, Li HM, Li L, et al. Anti-tuberculosis drug resistance patterns and trends in a tuberculosis referral hospital, 1997-2009. Epidemiology and Infection. 2011 Dec;139(12):1909-18.

42 Zar HJ, Udwadia ZF. Advances in tuberculosis 2011-2012. Thorax. 2013 Mar;68(3):283-7.

43 Chang CW, Wu MH, Chuang PC, Jou R. Characteristics of multidrug-resistant Mycobacterium tuberculosis in Taiwan: a population-based study. Infection, genetics and evolution : journal of

molecular epidemiology and evolutionary genetics in infectious diseases. 2011 Apr;11(3):633-9.

44 Yew WW, Leung CC: Management of multidrug-resistant tuberculosis:

Update 2007. Respirology 2008, 13:21-46.

45 Liu CH, Li L, Chen Z, et al. Characteristics and Treatment Outcomes of Patients with MDR and XDR Tuberculosis in a TB Referral Hospital in Beijing: A 13-Year Experience. PloS one. 2011 Apr 29;6(4).

46 Deng Y, Wang Y, Wang J, et al. Laboratory-based Surveillance of Extensively Drug-Resistant Tuberculosis, China. Emerging infectious diseases. 2011 Mar;17(3):495-7.

47 Atun R, Raviglione M, Marais B, Zumla A. Tuberculosis control is crucial to achieve the MDGs. Lancet 2010; 376:940–1.

48 Hu Y, Mathema B, Wang W, Kreiswirth B, Jiang W, Xu B. Population-based investigation of fluoroquinolones resistant tuberculosis in rural eastern China. Tuberculosis. 2011 May;91(3):238-43.

49 Magee MJ, Bloss E, Shin SS, et al. Clinical characteristics, drug resistance, and treatment outcomes among tuberculosis patients with diabetes in Peru. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2013 Feb 21.

50 Streicher EM, Muller B, Chihota V, et al. Emergence and treatment of multidrug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis in South Africa. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2012 Jun;12(4):686-94.

51 Serpa JA, Teeter LD, Musser JM, Graviss EA. Tuberculosis disparity between US-born blacks and whites, Houston, Texas, USA. Emerging infectious diseases. 2009 Jun;15(6):899-904.

52 Faustini A, Hall AJ, Perucci CA: Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. Thorax 2006, 61:158-163.

53 Villemagne B, Crauste C, Flipo M, Baulard AR, Deprez B, Willand N. Tuberculosis: The drug development pipeline at a glance. European journal of medicinal chemistry. 2012 2012-May;51:1-16.

TABLES AND FIGURES

Table 1.Characteristics of patients and information for the 10 provinces in 2004 – 2006

Province	Year	Population (*million)	Average of age	Total cases	No. of new cases（%）	No. of previous treated case（%）	No. of Male cases（%）
Jilin	2004-2005	27.1	45.58	349	276(79.1)	73(20.9)	249(71.4)
Beijing	2004	15.4	45.99	108	91(84.3)	17(15.7)	66(61.1)
Zhejiang	2006	47.2	41.09	97	72(74.2)	15(25.8)	53(54.6)
Fujian	2004-2006	35.1	49.00	479	323(67.4)	156(32.6)	319(66.6)
Henan	2004-2005	97.2	49.37	98	65(66.3)	33(33.7)	65(66.3)
Hunan	2004	61.6	45.98	102	47(46.1)	55(53.9)	74(72.6)
Xinjiang	2005-2006	19.6	46.85	186	125(67.2)	61(32.8)	112(60.2)
Gansu	2004-2006	26.2	39.12	220	193(87.7)	27(12.3)	134(60.9)
Sichuan	2004-2005	86.5	45.98	106	56(52.8)	50(47.2)	78(73.6)
Guangxi	2006	28.5	51.63	209	140(67.0)	69(33.0)	147(70.3)
Total	2004-2006	444.4	46.49	1954	1388(71.5)	556(28.5)	1297(66.4)

Table 2.Drug Susceptibility to Four First-Line Anti-tuberculosis Drugs

Drug	New cases (n=1388)	Previous treated cases (n=556)	Total (n=1954)
Any resistance to INH	217(15.6)	205(36.9)	422(21.6)
Any resistance to RFP	184(13.3)	192(34.5)	376(19.2)
Any resistance to SM	237(17.1)	153(27.5)	390(19.9)
Any resistance to EMB	106(7.6)	117(21.0)	223(11.4)
Single first line drug-resistant	168(12.1)	51(9.2)	219(11.2)
Resistance to INH only	53(3.8)	21(3.8)	74(3.8)
Resistance to RFP only	25(1.8)	10(1.8)	35(1.8)
Resistance to SM only	72(5.2)	14(2.5)	86(4.4)
Resistance to EMB only	18(1.3)	6(1.1)	24(1.2)
Multiple drug-resistant	116(8.4)	164(29.5)	280(14.3)
INH+RFP	22(1.6)	33(5.9)	55(2.8)
INH+RFP+SM	32(2.3)	40(7.2)	72(3.7)
INH+RFP+EMB	4(0.3)	21(3.8)	25(1.3)
INH+RFP+SM+EMB	58(4.2)	70(12.6)	128(6.5)
Multiple drugs resistant	91(6.6)	38(6.8)	129(6.6)
INH+SM	36(2.6)	13(2.3)	49(2.5)
INH+EMB	6(0.4)	4(0.7)	10(0.5)

INH+SM+EMB	6(0.4)	3(0.5)	9(0.5)
RFP+SM	29(2.1)	5(0.9)	34(1.7)
RFP+EMB	10(0.7)	7(1.3)	17(0.9)
RFP+SM+EMB	3(0.2)	4(0.7)	7(0.4)
SM+EMB	1(0.1)	2(0.4)	3(0.2)

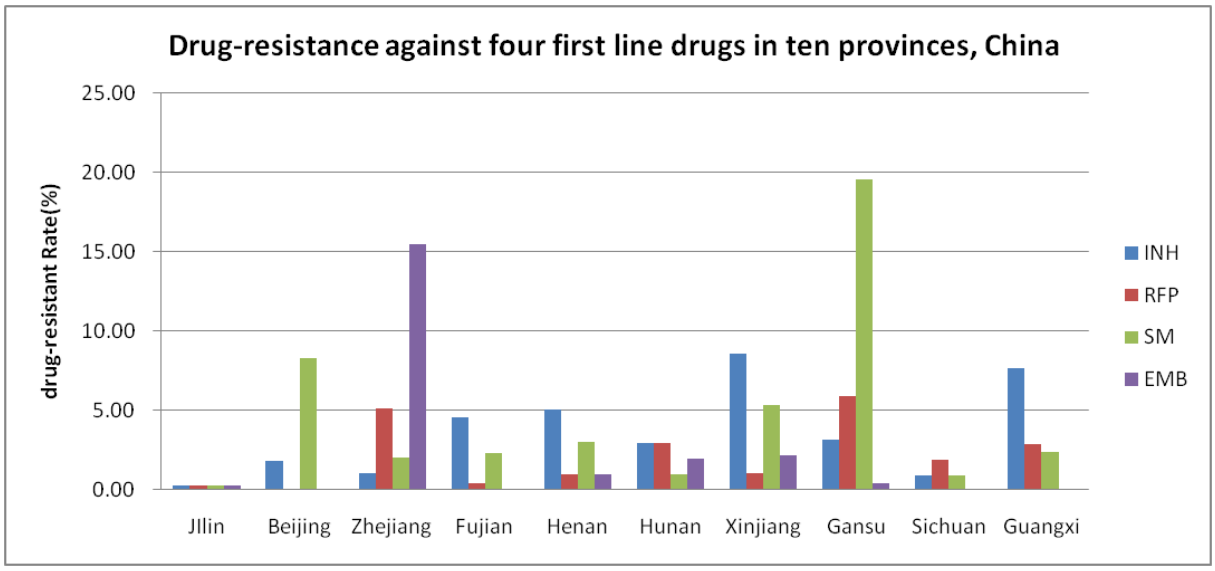
Table 3.Drug Resistance of TB for the 10 provinces in 2004 – 2006

	Jilin	Beijing	Zhejiang	Fujian	Henan	Hunan	Xinjiang	Gansu	Sichuan	Guangxi
INH	0.29	1.85	1.03	4.59	5.1	2.94	8.6	3.18	0.94	7.66
RFP	0.29	0	5.15	0.42	1.02	2.94	1.08	5.91	1.89	2.87
SM	0.29	8.33	2.06	2.3	3.06	0.98	5.38	19.55	0.94	2.39
EMB	0.29	0	15.46	0	1.02	1.96	2.15	0.45	0	0
SDR-TB	1.16	10.18	23.7	7.31	10.20	8.82	17.21	29.09	3.77	12.92
INH+RFP	0.57	1.85	0	2.71	0	5.88	2.15	0.91	15.09	4.78
INH+RFP+SM	1.15	1.85	1.03	6.68	2.04	5.88	2.15	2.27	11.32	1.91
INH+RFP+EMB	0.57	0.93	3.09	1.88	0	2.94	0	0.45	2.83	1.44
INH+RFP+SM+EMB	2.01	0	8.25	15.66	6.12	9.8	1.61	0.91	9.43	3.35
MDR-TB	4.30	4.63	12.37	26.93	8.16	24.51	5.91	4.55	38.68	11.48

Table 4.Analysis of Risk factor for Drug-resistant of TB in Patients

Characteristics	No. of Single DR((%)	OR (95% CI)	No. Of MDR(%)	OR (95% CI)
Gender				
Male	140（10.8）	Reference	193（14.9）	Reference
Female	77（12.5）	1.18（0.88-1.57）	86（14.0）	0.89（0.69-1.16）
TB treatment				
New cases	141(10.7)	Reference	112(8.5)	Reference
Previously treated cases	64(13.7)	1.48（1.08-2.04）	125(26.8)	5.46（4.12-7.24）
Age Group				
<15	4(30.8)	Reference	3(23.1)	Reference
15-24	26(10.4)	0.39（0.07-2.15）	22(8.8)	0.11（0.02-0.56）
25-34	35(8.7)	0.17（0.03-0.93）	70(17.4)	0.21（0.04-0.97）
35-44	38(10.7)	0.33（0.06-1.80）	54(15.2)	0.23（0.05-1.07）
45-54	29(9.8)	0.34（0.06-1.92）	42(14.5)	0.30（0.06-1.44）
>=55	66(12.4)	0.25（0.05-1.32）	83(15.5)	0.27（0.06-1.22）

Figure 1.The geographical location and relative area in selected 10 provinces



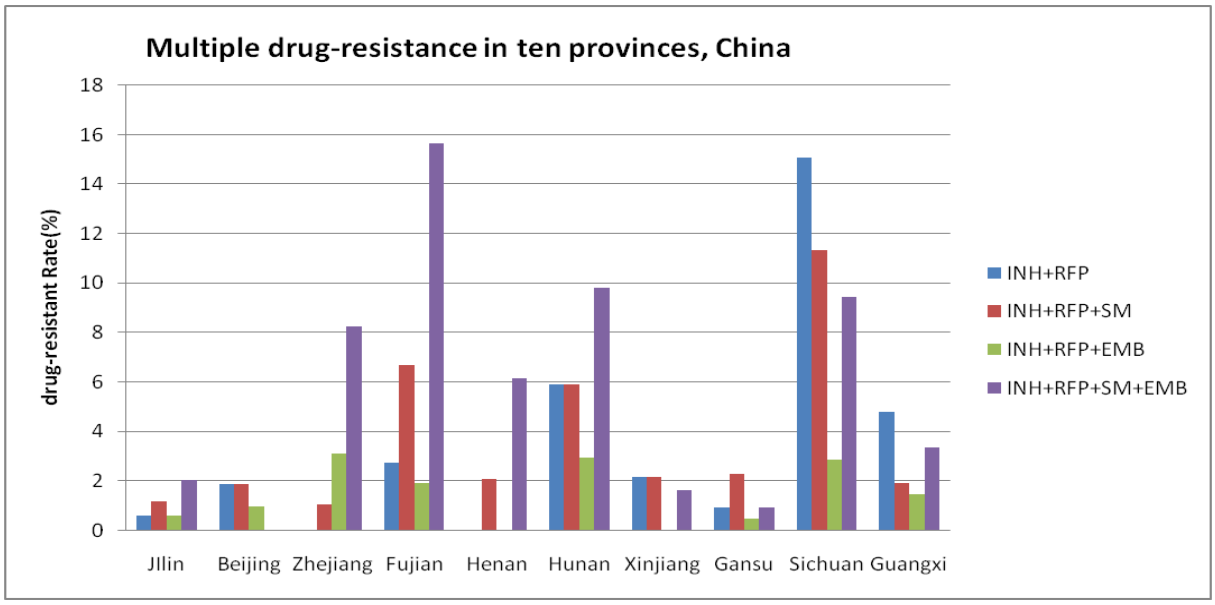


Figure 2.The Single Drug resistant for first-line drugs and MDR of TB compared in 10 provinces in 2004-2006