Study on drug resistance of *Mycobacterium tuberculosis* and its trend in Nantong.

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Abstract

Objective: This study aims to explore drug resistance of *Mycobacterium tuberculosis* (TB) and its trend in Nantong.

Methods: From January 2012 to December 2016, a total number of 1100 TB strains were collected from 1100 patients. To determine their drug resistant characteristics, all strains were treated with anti-tuberculosis drugs, including Ethambutol (EMB), Li Fuping (RFP), Isoniazid (INH) and Streptomycin (SM). Afterwards, the distribution of drug resistant strains in patients was analysed and the trend was profiled.

Results: Among those 1100 strains of *Mycobacterium tuberculosis*, 68 strains were identified as drug resistant, including 50 single-drug resistant strains and 18 multi-drug resistant strains; 23 strains were primary drug-resistant and 45 strains were acquired drug-resistant. The positive rate of *Mycobacterium tuberculosis* was 5.5% in 2012, 6.0% in 2013, 8.4% in 2014, and 7.3% in 2015 and 2.9% in 2016. As to specific drug sensitivity, 6 strains resistant to EMB, 10 strains resistant to RFP, 15 resistant to INH, 19 resistant to SM and 18 resistant to multiple drugs with the strains resistant to SM ranking the highest and the strains resistant to EMB the lowest. The drug resistant strains were related to 32 patients in youth group, 14 in middle-aged group and 22 in elder group. The rate of drug resistance in youth group is significantly higher than that of the middle-aged group and the elder group (P<0.05). In the sum of drug resistance strains there were 50 male patients and 18 female patients with the drug resistance rate of positive *Mycobacterium tuberculosis* strains of males significantly higher than that of females of statistical significance (P<0.05).

Conclusion: The drug resistance of *Mycobacterium tuberculosis* in Nantong area is high and more attention should be paid in this regard.

Keywords: *Mycobacterium tuberculosis*, Drug resistance, Drug sensitivity test, Drug resistance trend.

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Introduction

Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (MTB). Tuberculosis generally affects the lungs, but can also affect other parts of the body. TB spreads through the air when people who have active TB in their lungs cough, spit, speak, or sneeze. Anti-tuberculosis therapy is a common treatment for tuberculosis with therapeutic result directly affected by drug resistance of *Mycobacterium tuberculosis* [1]. However, drug resistance is a growing problem with increasing rates of multiple drug-resistant tuberculosis. To strengthen drug resistance analysis of *Mycobacterium tuberculosis*, summarize the drug resistance trend of tuberculosis and conduct specific control with active prevention of drug resistant strains becomes the focus of current clinical practices. In addition, it is also the key to ensure the therapeutic effect of TB drugs and reduce economic losses for the patients. In our study, 1100 tuberculosis positive patients were collected to implement drug resistance testing, followed by analysis of drug resistance trend. The retrospective analysis of specific test process is performed as follows.

Materials and Methods

General information

From January 2012 to December 2016, 1100 tuberculosis positive patients involved in our study were collected in Nantong Sixth People's Hospital. Inclusion criteria: positive sputum bacillus culture result is detected in the sputum test; patients are infected with humanoid *Mycobacterium tuberculosis*; voluntary patients are consented by medical ethics association of the hospital. Exclusion criteria: patients with other serious diseases in important organs, such as heart, kidney, spleen and stomach; patients with the implementation of treatment; patients with serious lung disease or infectious diseases. In the selected cases, there are 672 males and 428 females, age from 18 to 85 with the average age of 53.4 (sd=8.7) and the course of 6 months-7 y, 3.4 y on the average

(sd=2.2); 318 young cases (18-39 y old), 412 mid-age patients (40-59 y old) and 380 elder cases (60-85 y old).

Methods

The sputum smear was used to detect the drug resistance of *Mycobacterium tuberculosis* strains and all drug resistant strains were examined by anti-tuberculosis drug sensitivity test. Sputum smear examination: 3 sputum specimens were collected with 3-5 ml for each to make sputum smear and perform conventional acid fast stain followed by the observation under a microscope. The positive samples were separated out to culture positive strains. Strain culture: modified acidic Roche (L-J) medium was applied to culture strains; 4% sodium hydroxide was used to carry out pretreatment for the positive samples followed by inoculation of the treated specimens into culture medium on the condition of 37°C. Process of inoculation and cultivation should be operated in strict accordance with items of the Laboratory Science procedure of diagnostic bacteriology in tuberculosis. The growth of strains 3-7 d after inoculation was observed and the growing strains should firstly receive acid-fast strain and strains identification with the positive strains of sputum *Mycobacterium tuberculosis* given drug resistance test. Identification of strains: the growing strains were identified according to preliminary identification method of PNB/TCH and typing of culture characteristics, methods of high performance liquid chromatography and 16SrDNA sequencing were applied to make assisting identification. Methods of drug sensitivity test: strains of positive sputum *Mycobacterium tuberculosis* were treated with the detection of drug resistance and all strains of drug resistance were given drug sensitivity test of such related anti-tuberculosis drugs as Ethambutol (EMB), Li Fuping (RFP), Isoniazid (INH) and Streptomycin (SM) with the concentration of 2.0 μg/ml for the EMB drug sensitivity test, 40 μg/ml for the RFP test, 0.2 μg/ml for the INH test and 4.0 μg/ml for the SM test [2].

Observation index

Statistical analysis of drug resistance of *Mycobacterium tuberculosis*. Drug resistance is divided into primary resistance (cases without anti TB drug treatment or with the time of drug treatment less than 1 month) and acquired resistance (cases with the time of the drug treatment no less than 1 month). Cases resistant to both R and H or with drug resistance of more than two above drugs were classified as multidrug-resistant TB [3,4].

Statistical methods

SPSS19.0 software was used for data processing and analysis; the status of drug resistance was described by percentage and chi-square test was applied of statistical significance, P<0.05.

Results

Analysis of drug resistance of positive Mycobacterium tuberculosis strains

Among 1100 *Mycobacterium tuberculosis* strains, 68 strains were drug resistant, including 50 cases of single-drug resistance and 18 cases of multi-drug resistance. 23 strains were primary drug-resistant and 45 strains were acquired resistant. The rate of acquired resistant strains (4.1%) is significantly higher than that of primary drug-resistant strains (2.1%). The difference is significant, (x^2=7.345, P<0.05).

Analysis of drug resistance of Mycobacterium tuberculosis in different years

The positive rate of *Mycobacterium tuberculosis* was the highest in 2014 and lowest in 2016 with the drug resistance of *Mycobacterium tuberculosis* in specific years as shown in Table 1.

Table 1. *Analysis of drug resistance of Mycobacterium tuberculosis in different years.*

Year	Positive strains	Drug resistance	Multi-drug resistance	Drug resistance rate (%)
2012	217	12	3	5.5
2013	200	12	4	6.0
2014	274	23	7	8.4
2015	205	15	3	7.3
2016	204	6	1	2.9
Sum	1100	68	18	6.2

Analysis of drug resistance status in different strains

In 1100 cases of positive *Mycobacterium tuberculosis* there was highest rate of the strains resistant to SM (1.7%) and the lowest rate of the strains resistant to EMB (0.5%) with the details shown in Table 2.

Table 2. *Drug resistance status of resistant strains.*

Types of drugs	Strains	Drug resistance rate (%)
EMB	6	0.5
RFP	10	0.9
INH	15	1.4
SM	19	1.7
Multi-drug resistance	18	1.6

Sum	68	6.2

Analysis of the drug resistance in groups of different age

The drug resistance rate of positive strains of sputum *Mycobacterium tuberculosis* in the youth group was significantly higher than that of the middle-aged group and the

elderly group of statistical significance, x^2=13.502, 4.429 (P<0.05).

There was no significant difference in the drug resistance rate of positive strains of sputum *Mycobacterium tuberculosis* between the elderly group and the middle-aged group of no statistical significance, x^2=2.606 (P>0.05, Table 3).

Table 3. *Analysis on the drug resistance of positive strains in groups of different age.*

Group	Case	EMB	RFP	INH	SM	Multi-drug resistance	Total drug resistance rate
Youth	318	4 (1.3)	5 (1.6)	8 (2.5)	11 (3.5)	4 (1.3)	32 (10.1)
Middle-aged	412	1 (0.2)	3 (0.7)	3 (0.7)	2 (0.5)	5 (1.2)	14 (3.4)
Elder	380	1 (0.3)	2 (0.5)	4 (1.1)	6 (1.6)	9 (2.4)	22 (5.8)

Analysis of drug resistance of positive strains in groups of different gender

The total drug resistance rate of positive strains in the male group was significantly higher than that in the female group of statistical significance (P<0.05, Table 4).

Table 4. *Analysis of drug resistance of positive strains in groups of different gender.*

Gender	Case	EMB	RFP	INH	SM	Multi-drug resistance	Total drug resistance rate
Male	672	5 (0.7)	8 (1.2)	12 (1.8)	11 (1.6)	14 (2.1)	50 (7.4)
Female	428	1 (0.2)	2 (0.5)	3 (0.7)	8 (1.9)	4 (0.9)	18 (4.2)
x^2	--	1.256	4.518	2.288	0.083	2.144	4.718
P	---	>0.05	>0.05	>0.05	>0.05	>0.05	<0.05

Discussion

Tuberculosis (TB) is one of moderately common chronic infectious diseases in clinical trials, mainly resulting from *Mycobacterium tuberculosis* infection. The disease is likely to invade various body organs and cause multiple organ injury, thus posing a serious threat to life safety of the patients [5]. Anti-tuberculosis drugs are commonly used in the clinical treatment of tuberculosis, but the strains of drug resistant to *Mycobacterium tuberculosis* keep emerging with increasing abuse of antibiotics, which decreases therapeutic effect of some anti-tuberculosis drugs and effects prognosis of the patients [6-8].

The current clinical research results confirm that the drug resistance rate of patients with tuberculosis is increasing and related statistical results show that there are 5%-15% of patients with pulmonary tuberculosis with multi drug resistance [9,10]. Therefore, the key to improving the prognosis of patients turns out to strengthen the control of drug resistant strains and timely choose appropriate treatment plan

according to the actual drug resistance status [11]. In this study, the drug resistance rate of positive *Mycobacterium tuberculosis* strains in Nantong reached 6.2% in recent 5 y with many more acquired resistant strains, to which attention should be paid in clinical practices. The resistance rate of strains showed firstly an increasing trend and then a downward trend with the development of the years, which may be closely related to stricter control of antibiotics use in recent years. The results of drug resistance analysis showed that the rate of drug resistant to SM and INH was higher in terms of the single drug resistance, which may result from the fact that these two kinds of drugs are most widely used in the clinical trials and would be frequently applied in the early treatment of tuberculosis [12-14]. Drug resistance rate results of groups with different age showed that the resistance rate of the youth group was significantly higher than other two groups with the reasons as follows: the youth group had better organism's immunity and regulative capability and achieved more significant effects when receiving anti-tuberculosis treatment. Clinical symptoms turned out to be obviously improved after a period with the treatment, which made many patients fail to pay enough attention to regularly keeping taking medication with frequent interruption of treatment, easily leading to the emergence of drug-resistant bacteria [15-17]. Besides, the drug resistance rate of male patients was significantly higher than that of female patients, which may attribute to the difference between men and women in lifestyle and living habits and such poorer habits of the male as smoking, drinking, as well as staying up late all may result in the occurrence of drug-resistant bacteria [18,19].

In summary, the status of TB drug resistance in Nantong remains to be attached importance and the youth as well as the male are at high risk of suffering resistant bacteria. Education of related knowledge about TB disease should be strengthened in clinical practices and the patients should be informed the importance of strict medicine taking in accordance with the doctor's advice. Clinical medication requires to be standardized to actively prevent and control the emergence of drug-resistant *Mycobacterium tuberculosis* strains.

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