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DRUG DISCOVERY AND RESISTANCE

Prevalence of drug resistant *Mycobacterium tuberculosis* among children in China

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SUMMARY

The available data on the epidemic of drug resistant tuberculosis (TB) among children in China is limited. This study attempted to clarify the drug resistance profiles of clinical strains isolated from children and estimate risk factors related to acquisition of drug resistance. All *Mycobacterium tuberculosis* strains from children (age <15 years) and adolescent (age 15–18 years) TB patients received in the strain library of Chinese Center for Disease Control and Prevention between January 2005 and December 2012 were included in the study. A study collection included 450 clinical isolates (100 from children, 159 from adolescents, and 191 from adults) from all over China. Drug susceptibility testing was performed by a proportion method. As a result, the drug resistance and multi-drug resistance (MDR) rates in children were 55% (55/100) and 22% (22/100), respectively. In children with MDR-TB, new cases accounted for 40.9% (9/22). Compared with adults, the drug resistance rates were similar in all subgroups (new cases, previously treated cases and all cases) of children ($P > 0.05$), except for the lower resistance rate to isoniazid in total cases of children ($P = 0.011$). Patient related information was included in the MDR-TB association analysis. The treatment history was found to be strongly associated with MDR-TB in all three age groups ($P < 0.05$). Our results demonstrate that the prevalence of drug resistant TB in children in China is alarmingly high and similar to that seen in adults. In contrast, in adolescents, the drug resistance rate to most tested drugs was lower than in adults. Primary transmission and inadequate treatment are two equally important factors for the high MDR-TB rate in children. Thus, major efforts in the TB control in children should focus on decreasing the transmission of drug resistant TB and early testing of drug resistance.

1. Introduction

The emergence of drug resistant tuberculosis (TB), especially multi-drug resistant (MDR) and extensively drug resistant (XDR) *Mycobacterium tuberculosis* strains and their dissemination, poses a major threat to global TB control. According to the World Health Organization (WHO) Global Tuberculosis report 2012 [1], there were an estimated 310,000 MDR tuberculosis among notified patients with pulmonary tuberculosis in 2011. Even worse, almost 60% of these cases were in India, China and the Russian Federation.

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China is one of the high-burden countries, ranking the second of the total number of TB cases globally [1]. In 2007, the national survey of drug resistant TB in China was conducted. As a result, 5.7% of new cases of TB and 25.6% of previously treated cases had MDR-TB, indicating the serious epidemic of drug resistant TB in China [2].

Childhood TB remains a major but often unrecognized cause of disease and death among children in areas with high prevalence of TB [3,4]. Although pediatric cases reflect the ongoing transmission within communities, and were estimated as 10–15% of the total burden, little is known about the incidence of drug resistant TB in children. In the WHO Global TB report 2012, there were an estimated 0.5 million TB cases and 64000 deaths among children in 2011 [1]. In the 37 countries providing MDR-TB data on children, they accounted for 1–13% of total enrollments [1]. This was also the first time when estimates of TB burden among children were included in the global TB report. However, due to the diagnostic challenges and a lack of reporting data, this number was considered to be underestimated, especially in the areas with high burden of TB.

In this study, we investigated the TB cases including children (less than 15 years old), adolescents (15–18 years old) and matched adults (more than 18 years old) from all over China, a hotspot area of drug resistant TB. The rates of anti-TB drug resistance in different age groups were compared, and the risk factors related to development of drug resistant TB, especially MDR-TB, were identified.

2. Materials and methods

2.1. Study sample

M. tuberculosis isolates were selected from the *M. tuberculosis* strain library of Chinese Center for Disease Control and Prevention. The clinical strains in the library were discontinuously collected between January 2005 and December 2012. They were isolated from body fluid samples (sputum, bronchoalveolar lavage fluid, cerebrospinal fluid, pleural effusion, blood, or gastric juice) of confirmed TB patients admitted at institutions for TB control and cure, as well as TB hospitals in the following 25 provinces, municipalities and autonomous regions across China: Anhui, Beijing, Chongqing, Fujian, Gansu, Guangxi, Guizhou, Hebei, Henan, Hunan, Hubei, Heilongjiang, Inner Mongolia, Jilin, Jiangsu, Jiangxi, Liaoning, Qinghai, Shandong, Shaanxi, Shanxi, Sichuan, Tianjin, Xinjiang, Xizang (Tibet). All the *M. tuberculosis* strains from patients aged no more than 18 years were eligible for this study. The other group of strains from patients aged more than 18 years was selected using random number table to be matched by the isolation region and time. Demographic, epidemiologic and clinical information was obtained from the medical records of all patients, including gender, age, residence area, previous treatment history, medical facility, contact data, BCG vaccination, as well as disease type. New cases were patients diagnosed with TB that had never been treated with TB drugs or that had been treated for less than 1 month. Previously treated cases were patients who had been treated for TB for 1 month or longer.

2.2. Drug susceptibility testing

The chosen strains were recultured on Lowenstein-Jensen medium for 4 weeks at 37 °C. Drug susceptibility testing to four first-line anti-TB drugs [isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and streptomycin (STR)] and two second-line drugs [ofloxacin (OFLX) and kanamycin (KM)] was performed by a proportion method as recommended by WHO [5]. The concentrations of different drugs in the media were as follows: INH 0.2 μg/mL, RIF 40 μg/mL, EMB 2 μg/mL, STR 4 μg/mL, OFLX 2 μg/mL, KM 30 μg/mL. The strain was considered resistant to a specific drug when the growth rate was more than 1% compared to the control.

Any drug resistance was defined as resistance to at least one drug among six aforementioned anti-TB drugs. MDR was defined as resistance to at least INH and RIF. XDR was defined as resistance to at least INH, RIF, OFLX and KM. Pre-XDR was defined as resistance to INH and RIF and either OFLX or KM but not both [6].

2.3. Statistical analysis

IBM SPSS Statistics version 19.0 was used for statistical analysis. P values less than 0.05 were considered statistically significant. Comparison of the proportion of drug-resistant TB in different groups was done by chi-square test. The unadjusted odd ratio (OR) was used to evaluate univariate risk factors associated with MDR-TB. Variables with a P value less than 0.05 in the univariate analysis were used in the multivariable logistic regression analysis.

3. Results

3.1. Characteristics of patients studied

A total of 450 isolates were studied, including 100 from children (age <15 years), 159 from adolescents (age 15–18 years), and 191 from adults (age >18 years). The geographic distribution of enrolled TB patients is shown in Figure 1. The average age was 6.8 yr, 16.9 yr, and 40.8 yr in the above three groups, respectively. Male patients accounted for 57.0% (57/100) in children, 52.2% (83/159) in adolescents, and 63.9% (122/191) in adults. The proportion of new cases in three groups was 77.0% (77/100), 69.8% (111/159) and 68.1% (130/191), respectively. There was no significant difference in sex and treatment history among three age groups.

3.2. Rate of drug resistant TB

In this study, 54.4% (245/450) of the total samples were any drug resistant TB. The overall MDR rate was 25.8% (116/450), reflecting the gravity of situation with drug resistant TB in China.

Of the 100 isolates from children, 55.0% (55/100) were any drug-resistant TB, 22.0% (22/100) were MDR-TB (Table 1). Among the children with MDR-TB, new cases accounted for 40.9% (9/22). The resistance rate of MDR-TB to EMB, SM, OFLX and KM was 59.1%, 86.4%, 31.8%, and 9.1%, respectively. The XDR-TB was relatively rare in children patients (1%), however, that single XDR-TB case was a new one.

The drug resistance level of clinical MTB isolates in children was compared with that in adults (Figure 2). As a result, resistance to INH was significantly lower in children patients ($P = 0.011$). The INH resistance was further compared in new cases and previously treated cases between children and adults. INH resistance rate was lower in children at marginal level in new cases ($P = 0.054$), and non-significant in previously treated cases ($P = 0.439$). Besides, there was no significant difference in the drug resistance rate for the other five drugs, any drug, MDR, pre-XDR and XDR between children and adults ($P > 0.05$).

The drug resistance level of TB in adolescents was also compared with that in adults (Figure 2). It was observed that in the total cases, the resistance level to INH, RIF, SM and any drug was significantly lower in adolescents ($P < 0.05$). In the new cases, the resistance rate to INH, RIF, SM and MDR was significantly lower in adolescents ($P < 0.05$). In the previously treated cases, the resistance level to most drugs was lower in adolescents, but the difference was significant only for any drug resistance ($P = 0.022$). The resistance rate to EMB and OFLX was slightly higher in adolescents