

Short communication

# Primary and acquired drug resistance in childhood tuberculosis

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## المقاومة الأولية والمكتسبة للأدوية بين الأطفال المصابين بالسل

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**الخلاصة:** في دراسة أجريت بالمعهد الوطني الإيراني لبحوث السل وأمراض الرئة، في الحقبة الممتدة من عام 1999 إلى عام 2004، تم تحديد نمط مقاومة المتفطرة السلية لأربعة من أدوية الخط الأول المضادة للسل بين الأطفال المصابين بالسل الرئوي. وخلال مدة الدراسة كان هنالك 350 طفلاً إيجابياً المزرعة: سبعة منهم (2%) كانت لديهم مقاومة لواحد على الأقل من الأدوية الأربعة المضادة للسل. واكتُشفت المقاومة الأولية في أربعة حالات كما اكتُشفت المقاومة الثانوية في ثلاث حالات. وكانت معظم الحالات (ست حالات) بين اللاجئين الأفغان. كما كانت المقاومة الأولية والثانوية لدواء الريفامبيسين عالية، مما يدل على أن الأطفال في جمهورية إيران الإسلامية مهتدون بالإصابة بالسل المقاوم للأدوية.

**ABSTRACT** This study determined the resistance pattern of Mycobacterium tuberculosis to 4 first-line anti-tuberculosis drugs in children with pulmonary tuberculosis at the Iranian National Research Institute of Tuberculosis and Lung Diseases from 1999 to 2004. There were 350 children with positive cultures over the study period: 7 (2%) were resistant to at least one of the 4 anti-tuberculosis drugs. Primary resistance was detected in 4 cases and secondary resistance in 3 cases. Most cases (6) were among Afghan refugees. Resistance to rifampicin both in primary and secondary resistances was high, showing that children in the Islamic Republic of Iran face the threat of drug-resistant tuberculosis transmission.

Pharmacorésistance primaire et acquise dans la tuberculose de l'enfant

**RÉSUMÉ** La présente étude a déterminé le schéma de résistance de Mycobacterium tuberculosis à 4 médicaments antituberculeux de première intention chez des enfants atteints de tuberculose pulmonaire à l'Institut national iranien de recherche sur la tuberculose et les maladies pulmonaires de 1999 à 2004. Il y avait 350 enfants ayant des cultures positives durant la période étudiée : 7 (2 %) montraient une résistance à au moins un des 4 antituberculeux. Une résistance primaire a été détectée chez 4 cas et une résistance secondaire chez 3 cas. La plupart des cas (6) étaient des réfugiés afghans. La résistance à la rifampicine - primaire et secondaire - était élevée, ce qui montre que les enfants en République islamique d'Iran sont exposés à la menace de la transmission de la tuberculose pharmacorésistante.

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Received: 08/11/04; accepted: 12/05/05

## Introduction

Multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid and rifampicin, is an increasing health problem and a serious challenge for tuberculosis (TB) control programmes [1]. The drug-resistant *Mycobacterium tuberculosis* strains emerge whenever heavily infected individuals are inadequately treated, when treatment has been interrupted or when a single drug has been added to a failing regimen [2]. Children with MDR-TB usually have primary resistance and are infected with strains transmitted from adults with MDR-TB. The rate of transmission of strains of MDR-TB has been shown to be the same for children as for adults [3].

The frequency of TB drug resistance, especially MDR-TB, is generally high in countries categorized by the World Health Organization (WHO) as having poor control programmes [4,5]. Suboptimal control programmes can lead to rapid emergence of drug resistance not only in developing countries such as India [6] but also in populations in developed countries such as in New York city [7].

According to reports from South Africa, the incidence of isoniazid resistance was 5.6% and the rate of MDR-TB was 1% among children below 5 years of age [8]. Information about the susceptibility patterns of *M. tuberculosis* isolates against anti-TB drugs is an important aspect of TB control. Surveillance and analysis of local rates of TB drug resistance is helpful in the detection and monitoring of the extent of MDR strains, indicating the quality of TB control in a country [9]. However, there are few studies of drug-resistant TB in children and few data from developing countries.

The aim of this study was to determine the prevalence of drug resistance among children with TB at a referral hospital in

Tehran, Islamic Republic of Iran, and to compare the clinical and radiological features of drug-resistant and drug-susceptible TB among these children.

## Methods

### Setting

This retrospective study was conducted between January 1999 and August 2004 at the National Research Institute of Tuberculosis and Lung Disease, a referral centre for TB and lung diseases in Tehran. The DOTS [directly observed treatment, short-course] regimen has been used for all TB cases since 1995.

### Clinical data

The clinical records were reviewed for all children with positive cultures for *M. tuberculosis* who were admitted to the hospital over the study period. Data were collected regarding previous TB prophylaxis or treatment and whether they had close contact with an adult with pulmonary TB. The site of the infection was recorded. The results of tuberculin skin test (Mantoux test by intradermal injection; 0.1 mL of 5 tuberculin units) were noted. An induration of  $\geq 15$  mm after testing was regarded as significantly positive in accordance with WHO criteria [10]. Chest radiographs or computerized tomography scans were assessed and classified.

### Definition of primary and acquired resistance in *M. tuberculosis* isolates

Primary resistance was defined as the presence of resistance to one or more anti-TB drugs in strains obtained from patients who had never received treatment. Acquired resistance was defined as resistance to one or more anti-TB drugs in strains recovered

from patients who had received previous anti-TB treatment [11]. MDR-TB was defined as a condition in a child under the age of 15 years with findings of TB by radiography and a household contact with MDR-TB or culture demonstrating *M. tuberculosis*, with resistance to at least isoniazid and rifampicin by the proportion technique [12].

### Drug susceptibility testing

Sputum or gastric washing specimens were obtained from all children. All positive cultures are routinely sent to the National Research Institute of Tuberculosis and Lung Disease laboratory for sensitivity testing to isoniazid, rifampicin, streptomycin and ethambutol. Cultures were performed on Lowenstein–Jensen medium. Human immunodeficiency virus (HIV) testing was performed on all cases.

Laboratory procedures for determining drug resistance were as follows: specimens were digested and decontaminated by N-acetyl-L-cysteine NaOH method, as described by Kent and Kubica [13], with a final NaOH concentration of 1%. After decontamination, smears were prepared from the sediments for Ziehl–Neelsen acid fast staining. Then 0.2 mL of the processed specimen was inoculated onto each of 4 Lowenstein–Jensen slants (prepared in the local laboratory). All inoculated media were incubated at 37 °C. The inoculated solid Lowenstein–Jensen medium was inspected weekly for 8 weeks. All positive results were verified by Ziehl–Neelsen staining. Colonies from the surface of Lowenstein–Jensen medium were transferred into sterile test tubes containing 6–8 glass beads and 3.0 mL of Middlebrook 7H9 broth. The suspension was adjusted to 1 McFarland standard. Thereafter, dilutions of 1/10, 1/1000 and 1/100 000 were prepared and inoculated into drug-containing media and controls. The drug concentrations were as follows:

0.2 µg/mL isoniazid, 40 µg/mL rifampicin, 2 µg/mL ethambutol and 4 µg/mL streptomycin by the proportion method [14].

## Results

During the 5-year period from January 1999 to August 2004, 350 children with TB were admitted to the National Research Institute of Tuberculosis and Lung Disease. Among these, 7 children with MDR-TB were detected: 5 boys and 2 girls with a median age of 14.5 years (range 14–15 years). This give a frequency of MDR-TB resistance of 2% among children admitted to our centre. HIV tests were negative for all 7 cases. All the children had pulmonary TB and no evidence of extra-pulmonary involvement was found. The majority of cases were among Afghan immigrants: 6 children compared with only 1 child of Iranian nationality.

### Clinical and radiographic data

We reviewed the records of all 7 children. The most common symptom was productive cough found in 6 children (86%). Other symptoms were fever in 5 children (71%), haemoptysis in 3 (43%), dyspnoea and night chills in 2 (29%) and weight loss in 1 child (14%).

The radiographic changes in the 7 cases were reviewed. Among the 6 patients for whom radiographic results were available, the most common finding was infiltration in the lung parenchyma which was observed in 4 cases (57%). Along with calcification, cavitation, hilar adenopathy, pneumothorax, fibrosis and reticulonodular changes each in 1 case (14%).

### Susceptibility results

Drug susceptibility results showed that out of 7 cases, 6 (86%) had resistance to rifampicin, 5 (71%) to isoniazid, 4 (57%) to

streptomycin and 2 (29%) to ethambutol. In addition 2 cases (29%) had resistance to all 4 drugs (rifampicin, isoniazid, streptomycin, ethambutol), 1 case (14%) to isoniazid, rifampicin, streptomycin and 2 (29%) to isoniazid, rifampicin, 1 case (14%) had resistance only to streptomycin and 1 case (14%) had resistance only to rifampicin.

Among the 7 children, 6 (86%) had a history of a household TB contact. Only the 1 Afghan boy (14%) with resistance to isoniazid and rifampicin did not have any close contact with other TB patients. The most common TB contact was the mother and the uncle (2 cases, 29%). Contact with their brother and father was seen in 1 case (14%).

The drug resistance pattern showed primary resistance in 4 cases (57%) and secondary resistance in 3 cases (43%). The tests for primary resistance showed that 3 cases (77%) had resistance to rifampicin, 3 (77%) to isoniazid, 2 (50%) to streptomycin and 1 case (25%) had positive resistance to ethambutol. In the secondary resistance group, 3 cases (100%) had resistance to rifampicin, 2 (66%) to isoniazid, 2 (66%) to streptomycin and 1 case (33%) had positive resistance to ethambutol.

## Discussion

Surveillance of anti-TB drug resistance in a community provides a measure of the success of the national TB programme and gives an indication of suitable drug regimens for future use [15]. Primary or initial resistance patterns reflect transmission of drug-resistant strains, but as TB in children is a mark of recent TB transmission in a community, the frequency of drug resistance in children, particularly those < 5 years of age, reflects a more precise evaluation of the current situation [1,15].

The most comprehensive study to date was conducted over a 24-year period (1961–1984) in Kings County Hospital Center, New York [16,17]. Of 374 children screened for primary drug resistance, 16.3% had resistance to 1 or more anti-TB drugs. Isoniazid resistance was relatively stable at 10%, but for rifampicin, which was introduced during this period, resistance was on the increase [16].

In another study in the year 2000, out of 306 children with positive culture for *M. tuberculosis*, 6.9% were resistant to isoniazid and 3.2% had MDR-TB [18]. Research on 11 480 children with TB in the United States during 1993–2001 found that among culture-positive children, drug resistance to isoniazid and rifampicin was 7.3% and 1.6% respectively [19].

In developing countries, there are no comprehensive studies on the prevalence of drug resistance in children, although some reports of the status of drug resistance among the adult population are available. In the year 2001, a study was conducted by Al-Marri in Qatar [20]. In this investigation, out of 406 cases with a positive culture for *M. tuberculosis*, at least 15% had resistance to 1 of the 4 anti-TB drugs. Out of this, 95% had primary and 5% had secondary resistance. Also, maximum resistance was observed for isoniazid in 15%. In our study out of 350 children, 7 cases had resistance to at least 1 of the 4 anti-TB drugs and 5 (1.4%) had MDR-TB. The frequency of MDR-TB in TB cases admitted to our centre was 2%, which is lower than similar data and studies reported by Al-Marri [20]. A low prevalence in a retrospective study could be due the likelihood of missing cases compared with a prospective study; however, in our study, culture and antibiograms were performed in all TB cases reported to this centre. Since a comprehensive and

thorough study has not been performed in regard to drug resistance in children in the Islamic Republic of Iran, accurate data and figures for comparison do not exist. As mentioned in the results, out of 7 patients that had MDR-TB, 6 were Afghan immigrants. This high proportion of immigrants with MDR-TB could be explained by their low socioeconomic status, malnutrition, delay in diagnosis and irregular use of anti-TB medications, which act as predisposing factors in this group of patients.

Meanwhile, in 4 of the children (57%) primary resistance and in 3 (43%) secondary resistance was detected which could be explained by the fact that primary resistance is more common in children [3]. Several studies have reported a very low rate of resistance to rifampicin [3,21,22]. However in our study of MDR-TB, resistance to rifampicin both in cases of primary and secondary resistance was high. According to our results, 6 patients (85%) had resistance to rifampicin; 3 (42%) from each of

primary and secondary resistance groups. Also, with regard to isoniazid resistance, 3 (75%) from the primary and 2 (66%) from the secondary resistance group showed resistance. At the same time resistance to ethambutol and streptomycin in both groups were similar. Different circumstances such as genetic factors in Iranian and Afghan children and/or the composition of anti-TB drugs available in the Islamic Republic of Iran might be responsible for the high rate of rifampicin resistance observed. In addition, the National Research Institute of Tuberculosis and Lung Disease is a referral centre, thus the findings of a high rate of resistance to rifampicin cannot be extrapolated to the whole society.

It is recommended that more detailed culture and antibiogram studies should be conducted in children with TB, especially in those who have clinical and/or radiological evidence of drug resistance, and continued measures are needed to control the spread of drug resistance in our society.

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