

## Short Communication

# The Resistance to Major Antituberculous Drugs of *Mycobacterium tuberculosis* Strains Isolated from the Respiratory System Specimens of Tuberculosis Patients in Duzce, Turkey

C. Elif Ozturk\*, Oner A. Balbay<sup>1</sup>, Demet Kaya, Ismail Ceyhan<sup>2</sup>, Ismet Bulut<sup>1</sup> and Idris Sahin

*Department of Microbiology and <sup>1</sup>Department of Chest Diseases, Abant Izzet Baysal University, Düzce School of Medicine, Duzce 81620 and <sup>2</sup>Tuberculosis Reference and Research Laboratory, Refik Saydam Hygiene Center, Republic of Turkey Ministry of Health, Ankara 06490, Turkey*

(Received May 26, 2004. Accepted October 20, 2004)

**SUMMARY:** Though generally curable, tuberculosis (TB) is becoming increasingly resistant to commonly used antibiotics. Drug-resistant and multidrug-resistant (MDR)-TB is a consequence of monotherapy, insufficient drug therapy and national TB control programs. The present study was designed to reveal the resistance to major antimicrobial drugs (isoniazid [INH], streptomycin [SM], ethambutol [EMB], and rifampicin [RIF]) of *Mycobacterium tuberculosis* isolated from the respiratory specimens of TB patients in Duzce, Turkey. A total of 62 TB patients (46 male, 16 female; age: 17-75 mean: 42 ± 15.9) were included in the study; 52 (83.8%) were new cases and susceptible to all anti-TB drugs, while 10 (16.2%) were previously treated cases. Antimicrobial susceptibility tests were performed by the proportion method in Löwenstein-Jensen medium. Fifty-two of the 62 (83.8%) isolated *M. tuberculosis* strains were found to be susceptible to all drugs, and 7 (11.3%), 5 (8%), and 3 (4.8%) were resistant to SM, INH, and RIF, respectively; 3 (4.8%) were MDR. There were no EMB-resistant strains. The results of this study show the presence of drug-resistant and MDR strains of TB at Duzce in the northwest part of Turkey.

*Mycobacterium tuberculosis* infects one-third of the world's population (1), and tuberculosis (TB) still accounts for a large number of deaths and great morbidity worldwide. The disease, while generally curable, is becoming increasingly resistant to commonly used antibiotics (2). The modern era of TB began in 1946 with a demonstration of the efficacy of streptomycin (SM). In 1952, the much more effective drug isoniazid (INH) became available, making TB curable in the great majority of patients, and in 1970, rifampicin (RIF) came to be recognized as at least equal to INH. Drug-resistant (DR)-TB has been reported since the early days of the introduction of chemotherapy, however, the global magnitude of DR-TB has not been well studied until recently (2). The detection of *M. tuberculosis* and its drug susceptibility pattern is increasingly being recognized as an important component of global TB control (3), and it is critical to test the drug susceptibility of TB bacilli in order to guide therapy (4). The first survey of drug resistance was released in 1998 and included data from 35 countries (5). In 1992, the Third World Congress on TB concluded that there was little recent information on the global magnitude of multidrug-resistant (MDR)-TB, defined as resistance to at least INH and RIF. According to the World Health Organization (WHO) report of 2004, the population of Turkey was 70,318,000, the total number of TB cases reported to the WHO was 18,043, and the case notification rate was 26 per 100,000 people (6). Based on the data collected from several local and regional studies, the emergence and rapid growth of MDR-TB are still a matter of

great concern. Because treatment approaches are often inappropriate, case finding is mainly passive, follow-up is inconsistent, the rates of treatment completion are low, and therapy is not directly observed. Thus, estimates of the overall prevalence and cure rate throughout the country cannot be made accurately (7).

In a recent study in Turkey, it was shown that primary resistance to INH and RIF is 8.6 and 5.6%, respectively, and acquired resistance is 25.3 and 26.5%, respectively, and the primary and secondary MDR rates were 3.1 and 18.5%, respectively (9). We conducted this study to determine drug resistance to anti-TB drugs among TB patients in the Duzce district in the northwest part of Turkey.

Sixty-two clinical *M. tuberculosis* strains were isolated from respiratory samples of TB patients between December of 2000 and February of 2004. Of these 62 patients, 46 (74%) were male and 16 (26%) were female, and they were aged between 17-75 years (mean: 42 ± 15.9). The respiratory samples were obtained in a sterile container in the morning. After decontamination, samples were stained by Ehrlich-Ziehl-Neelsen (EZN) for the presence of acid-fast bacilli, and inoculated to Löwenstein-Jensen (LJ) medium (Biomérieux, Marcy l'Etoile, France). All cultures were incubated at 37°C for 4 to 6 weeks. *M. tuberculosis* strains were differentiated from other mycobacteria by tests such as slow growing, the pigmentation properties of colonies, nitrate reduction, niacin accumulation, and heat-stable catalase tests.

Drug susceptibility tests were performed in the Tuberculosis Reference and Research Laboratory in the Refik Saydam Hygiene Center, Ankara. Resistance to anti-TB drugs was defined according to the proportional method (10) as greater than 1% (10% for SM) growth in the presence of critical drug concentrations with LJ medium. The critical drug

\*Corresponding author: Mailing address: Department of Microbiology, Abant Izzet Baysal University, Düzce School of Medicine, Konuralp-Duzce 81 620, Turkey. Tel: +90-380-541-41-22, Fax: +90-380-541-41-05, E-mail: elifozturk1968@yahoo.com

concentrations were the following: INH, 0.2  $\mu\text{g/ml}$ ; RIF, 40  $\mu\text{g/ml}$ ; SM, 4  $\mu\text{g/ml}$ ; ethambutol (EMB), 2  $\mu\text{g/ml}$ . *M. tuberculosis* H37Rv was used as a susceptible control strain.

We use the term “new case” here to refer to TB patients who have never received anti-TB drugs or who received them for no more than 1 month of treatment. The term “previously treated case” refers to patients who had received at least 1 month of anti-TB therapy in the past. Previously treated cases included relapse, treatment failure, patients returning after defaulting, and chronic cases. “MDR-TB” was defined as TB resistant to chemotherapy with at least INH and RIF (8). “Drug resistance among new cases (primary drug resistance)” is defined as the presence of resistant *M. tuberculosis* in new TB cases who, in response to direct questioning, deny having had previous anti-TB treatment or having been treated for more than 1 month with no evidence of any such treatment history. Patients who are diagnosed with TB and start anti-TB treatment and whose bacilli develop drug resistance to one or more of the medicines used during treatment are said to have developed “acquired (or secondary) drug resistance” (8). Newly diagnosed TB was treated with INH, RIF, pyrazinamide (PRZ) and EMB, and relapsed TB was treated with INH, RIF, PRZ, EMB, and SM. Patients were clinically judged to have completed treatment if they received anti-TB drugs for a minimum of 6 months and had no microbiologic or clinical evidence of TB during the last 3 months of treatment (6).

Fifty-two of 62 (83.8%) TB patients were new cases and susceptible to all anti-TB drugs, and 10 of 62 (16.2%) were previously treated cases. The characteristics of all cases are shown in Table 1. Seven (11.3%) of 62 cases, 5 (8%), and 3 (4.8%) were resistant to SM, INH, and RIF, respectively, and 3 (4.8%) were MDR. All RIF-resistant isolates were also found to be resistant to INH. There was no EMB resistance. The rate of resistant *M. tuberculosis* isolates is shown in Table 2 and the distribution of DR isolates is shown in Table 3. All new cases completed their 6-month anti-TB regimens with no relapse, and all previously treated cases completed their 8-month anti-TB regimens with one case of relapse. All MDR cases have been referred to a center which specializes in

Table 1. The ratio of drug resistance among new TB cases and previously treated cases

	No. of DS isolates (%)	No. of primary DR	No. of secondary DR (%)	Total
New cases	52 (83.8)	0	0	52
Previously treated cases	0	0	10 (16.2)	10
Total	52	0	10	62

DS, drug susceptible; DR, drug resistance.

Table 2. The rate of resistance to *M. tuberculosis* isolates

Drug	No. of resistant isolates/no. of total isolates	Drug resistance (%)
SM	7/62	11.3
INH	5/62	8
RIF	3/62	4.8
MDR	3/62	4.8
EMB	0/62	–

SM, streptomycin; INH, isoniazid; RIF, rifampicin; MDR, multidrug resistant; EMB, ethambutol.

Table 3. The distribution of drug resistance of *M. tuberculosis* strains

Sample no.	INH	RIF	SM
1	R	R	R
2	R	R	S
3	R	R	S
4	R	S	R
5	R	S	S
6	S	S	R
7	S	S	R
8	S	S	R
9	S	S	R
10	S	S	R

S, susceptible; R, resistant.

MDR-TB for follow up.

Drug resistance in TB has been reported since the early days of the introduction of chemotherapy (2). In the 1960s, primary resistance to at least one drug with a prevalence of 3 - 13% was reported in some European countries, the United States, and Japan (11-14). DR-TB is primarily a consequence of monotherapy, insufficient drug therapy and national TB control programs, or poor socio-economic environments, delayed diagnosis, inadequate treatment regimens, non-compliance by patients, and significant rates of nosocomial transmission. The first report of a global project on anti-TB drug resistance was warmly welcomed because it demonstrated that the joint efforts of the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) had been successful (15). The second report gives the results of a survey conducted in 58 different geographical settings between 1996 and 1999, 3 years after the first survey (35 settings) (8), for the purpose of collecting worldwide information on the DR of *M. tuberculosis*. The results of the second survey are satisfactory because they show no clear increase in the prevalence of MDR since the first survey: the median prevalence of MDR in strains isolated from new cases was 1.4% (range 0 to 14.4%) in the first survey and 1% (range 0 to 14.1%) in the second survey; and the median prevalence of MDR in strains isolated from previously treated cases was 13% (range 0 to 54%) in the first survey and 9.3% (range 0 to 48.2%) in the second survey (8). The available data suggest that globally, primary MDR-TB is not a problem (median 1% in 64 countries) (2). In 2000, the WHO reported that resistant to INH, RIF, EMB, and SM was 2.2 - 16.5, 0.3 - 5.3, 0 - 6.5, and 1.6 - 13.4%, respectively, and MDR-TB was found at a rate of 3.1 - 48.2% (15).

Over a 21-year study period in Turkey, the total resistance to INH, RIF, and SM were determined to be 10.5, 6.9, and 7.0%, respectively, and MDR-TB was found in 194 divided by 3,319 (5.8%) samples (16). Additionally, Saygan et al. reported the following rates of resistance in a set of 562 samples: INH, 94 (16.73%); SM, 68 (12.10%); EMB, 24 (4.3%); RIF, 89 (15.84%); and MDR-TB, 59 (10.5%) from all regional TB laboratories of Turkey (17). Kart et al. have also studied the resistance pattern of drugs in two cities of Turkey (Zonguldak and Kayseri) from 1992 to 1999, finding resistance levels to INH, RIF, SM, and EMB of 14.4, 10.6, 21.1, and 2.4%, respectively (18). These rates of DR and MDR in Turkey are consistent with those reported by the WHO as well as with those found in the present study. In conclusion, secondary DR-TB and MDR-TB are a matter of concern in the northwest part of Turkey as well as throughout the

country. Additional studies with larger groups of subjects are still needed.

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