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A STUDY OF *MYCOBACTERIUM TUBERCULOSIS* DRUG RESISTANCE IN PULMONARY TUBERCULOSIS

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ABSTRACT

Tuberculosis remains a major public health problem in both developing and developed countries. Drug-resistant tuberculosis is an increasing health problem and serious challenge to tuberculosis (TB) control programs. Information about the susceptibility pattern of *Mycobacterium tuberculosis* isolates against anti-tuberculosis drugs is an important aspect to TB control. The objectives of the study were to evaluate the frequency of drug-resistance and to survey the nature of drug resistance among patients with pulmonary tuberculosis.

Ninety-one *M. tuberculosis* strains were isolated from sputum samples of patients referred to Cerrhapasa Medical Faculty Hospital, Istanbul, Turkey, during a 9 month period. Drug susceptibility testing was performed to isoniazid (INH), streptomycin (SM), ethambutol (EMB) and rifampin (RMP) on Lowenstein-Jensen medium according to proportion method.

Total resistance was identified in 40 of 91 patients (44%). The highest rate of primary resistance was to SM (21.1%), followed by INH (15.8%), RMP (5.3%) and EMB (2.6%). Secondary resistance was most frequent to INH (33.3%), followed by SM (28.6%), RMP (23.8%) and EMB (14.3%). Multidrug resistance (MDR) was observed in 6 of the 91 cases (6.6%). Due to the high prevalence of drug resistance, particularly in developing countries, further studies should be conducted regularly to monitor resistance in these countries.
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INTRODUCTION

The burden of tuberculosis (TB) today is greatest in low-income and developing countries; over 90% of all cases arise there, and over 95% of deaths from the disease occur there.[1]

Drug-resistant TB is an important aspect of TB control. Drug resistance has been known since the discovery of the first anti-TB drug, streptomycin, in 1954 and the presence of resistant mutants in wild populations of mycobacteria has been well documented. Drug resistance in TB is classified into 2 types; primary, i.e. previously untreated patients who are found to have drug-resistant organisms, presumably because they have been infected from an outside source of resistant bacilli, and secondary, i.e. patients who initially have drug-susceptible tubercle bacilli that later become resistant because of inadequate, inappropriate or irregular treatment or, more importantly, because of nonadherence to treatment protocols.[1,2,3,4]

Information about susceptibility patterns of *Mycobacterium tuberculosis* drugs is very important for control of TB, and surveillance and analysis of local rates of TB drug resistance is helpful in the detection and monitor-

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ing of the extent of multidrug-resistant (MDR) tuberculosis strains (MDR, defined as resistance to at least isoniazid and rifampin), indicating the quality of TB control in the country.

Knowledge of the prevalence of primary drug resistance guides the selection of drugs used in the primary treatment of TB. Acquired resistance is believed to be closely related to efficacy of the treatment program, and early diagnosis of MDR is essential to prevent its transmission in the community.[3,4]

The aim of the present study was to evaluate the frequency of drug-resistance to isoniazid (INH), rifampin (RMP), ethambutol (EMB) and streptomycin (SM) in Istanbul, Turkey, during a 9 month period.

MATERIAL AND METHODS

This study involved patients with pulmonary tuberculosis referred to the Cerrhapasa Medical Faculty Hospital, Istanbul, Turkey, from January to September 1999. Bacteriologic examinations were perfomed at the Clinical Microbiology Laboratory of Cerrhapasa Medical Faculty of Istanbul University.

Ninety-one *Mycobacterium tuberculosis* strains were isolated from sputum samples of 91 pulmonary tuberculosis patients. The pattern of drug resistance was analysed from results of their sputum culture and sensitivity tests. After digestion and concentration of sputum specimens by 4% NaOH, a smear was made and stained by Ziehl-Neelsen (ZN) staining method. For each sample, 2 mL of the concentrated sputum specimens were inoculated into 2 slopes of Lowenstein-Jensen (LJ) culture medium. Slopes were inoculated at 35°C and examined weekly for growth for 8 weeks. Identification of a positive culture for *M. tuberculosis* was based on acid fastness, rate of growth, morphology, production of niacin and nitrate tests.[5,6]

Drug susceptibility testing was done on only one *M. tuberculosis* isolate for each patient. LJ medium was impregnated with necessary antibiotics according to the proportion.[5,6] Each strain was tested against four antibiotics according to the following concentrations:

Isoniazid (INH) 0.2 μg/mL
Rifampin (RMP) 2 μg/mL
Ethambutol (EMB) 2 μg/mL
Streptomycin (SM) 4 μg/mL

The critical proportion of resistant bacilli required to define a strain as resistant was 1% for all of the four tested drugs. Quality control was conducted on each batch of media, using reference sensitive strain H37RV.

Primary resistance was defined as the presence of resistance to one or more anti-tuberculosis drugs in patients who had never received previous anti-tuberculosis treatment. Total resistance was calculated by adding together the number of cases for each drug from each pattern of resistance. MDR referred to tuberculosis caused by *Mycobacterium tuberculosis* strains resistant to at least two antibiotics including INH and RMP.[3,5,6]

RESULTS

A total of 91 patients with culture positive pulmonary tuberculosis were evaluated. Of the 91 patients, 38 (41.8%) were new cases whilst 42 (46.2%) had a history of previous anti-tuberculosis treatment and 11 (12%) had insufficient data about treatment. Ninety-one *Mycobacterium tuberculosis* strains were isolated from an equal number of pulmonary tuberculosis patients.

The results of culture susceptibility testing are presented in Tables I and II. Of 91 *M. tuberculosis* studied strains, 51 (56%) were completely sensitive to the four anti-tuberculosis drugs and 40 (44%) were resistant to at least one drug (Table I). Resistance to only one drug

Table I: Susceptibility pattern of *M. tuberculosis* isolates in 91 studied patients.

	Primary resistance (No prior treatment) n=38 n (%)	**Secondary resistance (Prior treatment) n=42 n (%)**	**Unknown n=11 n(%)**	**Total n= 91 n(%)**
Sensitive to all drugs	26 (68.4)	18 (42.8)	7 (63.6)	51 (56)
Resistant to at least one drug	12 (31.6)	24 (57.2)	4 (36.4)	40 (44)
Resistant to : one drug	8 (21.1)	11 (26.2)	3 (27.3)	22 (24.2)
two drugs	3 (7.9)	7 (16.7)	1 (9.1)	11 (12.1)
three drugs	_	4 (9.5)	_	4 (4.4)
four drugs	1 (2.6)	2 (4.8)	_	3 (3.3)

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Table II: Primary and secondary resistances to anti-tuberculosis drugs.

Drug	Primary resistance (No prior treatment) n%	Secondary resistance (Prior treatment) n%	Unknown n%	Total n%
INH	6(15.8%)	14(33.3%)	1(9.1%)	21(23.1%)
RMP	2(5.3%)	10(23.8%)	_	12(13.2%)
EMB	1(2.6%)	6(14.3%)	_	7(7.7%)
SM	8(21.1%)	12(28.6%)	2(18.2%)	22(24.2%)

was the most common, and was observed in 22 strains (24.2%). Resistance to two drugs was seen in 11 isolates (21.1%), whereas resistance to 3 drugs was observed in 4 strains (4.4%) and to 4 drugs in 3 strains (3.3%) (Table I). Total primary and secondary resistance to at least one drug was found to be 31.6% and 57.2% respectively. Resistance to one drug was the most common in the two groups (Table I).

As Table II shows, in 91 *M. tuberculosis* isolates, total resistance to SM was the most frequent (24.2%), followed by INH (23.1%), RMP (13.2%) and EMB (7.7%). Of the 38 new pulmonary tuberculosis patients, primary resistances were found to SM, INH, RMP and EMB in 8 (21.1%), 6 (15.8%), 2 (5.3%) and 1 (2.6%) strains respectively. Of the 42 patients who had previously been treated for tuberculosis, secondary resistance was observed to INH, SM, RMP and EMB, in 14 (33.3%), 12 (28.6%), 10 (23.8%) and 6 (14.3%) strains respectively.

Of the 11 patients who had previous unknown treatment for TB, one case (9.1%) showed resistance to INH and 2 cases (18.2%) were found to be resistant to SM (Table II).

MDR was observed in 2.6% (1 strain) of new patients and in 11.9% (5 strains) of patients with prior treatments. The total resistance of MDR was 6.6% among 91 *Mycobacterium tuberculosis* strains.

DISCUSSION

Drug resistance is a major problem in the treatment of tuberculosis. Previous badly managed anti-tuberculosis treatment has been reported in the literature as a factor favoring drug resistance. The prevalence of resistance to anti-tuberculosis drugs was found to be higher in developing countries as compared to developed countries.[1,2,3,4,7,8,9,10] The total rate of anti-tuberculosis drug resistance in this study was found to be 44% (Table I). This prevalence rate was higher than those of studies in developed countries: 14.1% in 1991 in the USA,[7] 25.3% in 1990 and 10.6% in 1996 in Japan.[8] Several studies on anti-tuberculosis drug resistance in Turkey have been reported so far. Aysev reported the resistance rate as 29.6% in 4677 patients in Ankara for the years of 1985-1989.[9] Bengisun et al. reported a resistance rate of 39.2% in 3319 patients for the years of 1974-1997.[10] Tahaoglu and co-workers found a total resistance rate of 35.5% in 785 patients with pulmonary tuberculosis in 1992.[11] The total drug resistance in our study is similar to those encountered in some studies performed in different geographical areas of Iran. Moniri et al. reported the total resistance rate as 47.8% in Kashan.[12] Ghazi Saidi et al. found a total resistance rate of 35.8% in Tehran.[13] On the other hand, Heidarnajad and Nagili observed only 16.9% total resistance in 148 patients in a recent study in Tabriz.[14]

As evident from Table I, the data from the present study show that the rate of resistance to at least one drug was 24.2%. In the subgroups of 80 patients in whom data about previous treatment were available, the rate of resistance to at least one drug was 21.1% in never treated patients and 26.2% in previously treated patients. In the USA, a rate of resistance to at least one drug was reported to be 14.4% in 1991[15] whereas lower rates were reported in other European countries: 9.8% in England and Wales for the years 1982-1991,[3] 13.8% in Italy for the years 1992-1995,[3] and 6.7% in Switzerland for the years 1995-1996.[14] In Saudi Arabia the rate of resistance to at least one drug was reported to be 32% in 1992.[16] The one-drug resistance rate observed in this study (24.2%) was higher than that reported in the above mentioned countries, but it was lower than that reported in Saudi Arabia (Table I).

The total primary resistance rate of 31.6% found in the present study was higher than those reported for Western Europe, USA, and certain other countries (Table I).[16,17] Most of our isolates with primary drug resistance were resistant to one drug and most commonly to SM. In global surveillance data, most countries reported a higher prevalence of primary resistance to SM than to other

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antibiotics.[17] As suggested by others, one possible explanation for this finding is the frequent use of SM for other, non-mycobacterial conditions, including renal infection and infections in private sectors.[9,10]

In our study the most effective drug was found to be EMB with 2.6% primary resistance in non-prior treatment patients and 14.3% secondary resistance in prior treated patients (Table II). As expected, the prevalence of secondary resistance was higher than primary resistance. Primary resistance to INH and RMP was 15.8% and 5.3% respectively and secondary resistance to these drugs was found to be 33.3% and 23.8% respectively (Table II). Our results were comparable with those reported in other studies in Turkey.[9,10,11] The widespread use of INH and RMP in this country can probably explain the quite consistent primary and secondary resistance found toward INH and RMP. In collected data of 35 countries published by Pablos-Mendez, the mean primary resistance to INH, RMP and EMB was 7.3%, 1.8% and 1% respectively. In their report, resistance to INH or SM was the most common. Heidamejad and Nagili[14] encountered primary resistance to SM and INH in 12.8% and 7.4% respectively and no primary resistance to other drugs was found in Tabriz. Ghazi Saidi and co-workers reported primary resistance to SM 10.8%, to INH in 8.7%, to RMP in 3.2% and to EMB in 3%.[13] They reported secondary resistance of 40.5%, 31.5%, 27.9% and 16.7% to INH, RMP, SM and EMB respectively in Tehran.

In this study, MDRs were found to be 6.6% (6 out of 91 strains), thus primary and secondary resistance was 2.6% and 11.9% respectively. This frequency is in agreement with those reported in Turkey and some other studies.[9,10,11,16] Overall median prevalence of MDR was reported to be 2.2%, with a range from 0% (Kenya) to 22% (Lativa) (Portugal 3.7%, Peru 4.5%, Russia 7.3%, Argentina 8%).[17] Our data showed high MDR levels, similar to other developing countries.[17]

In conclusion, the data obtained from our study were not representative of TB drug resistance as a whole, but they gave a rough guide to anti-tuberculosis drug resistance rates in Turkey. This study showed that primary and secondary anti-tuberculosis drug resistance could be a problem in developing countries. Studies such as this investigation should be conducted regularly to monitor drug resistance in our country in order to effectively manage national tuberculosis control efforts.

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