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Susceptibility of *Mycobacterium tuberculosis* Strains to First-Line and Second-Line Antituberculosis Drugs in Ege University Hospital

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Abstract: Recently tuberculosis has shown a speedy worldwide spread. The incidence of drug-resistant *Mycobacterium tuberculosis* is increasing in almost all industrialized and developing countries. The epidemiology of multiple drug resistance varies in different regions and countries.

The aim of this study was to determine the activities of first-line (isoniazid, rifampin, ethambutol and streptomycin) and second-line (kanamycin, para-aminosalicylic acid, ethionamide and capreomycin) antituberculosis drugs on 100 various clinical isolates of *M. tuberculosis*. *Mycobacterium tuberculosis* ATCC 27294, ATCC 35838, ATCC 35825 and ATCC 35837 were used for internal quality control.

First-line drug resistant strains were isolated from 10 clinical specimens. Six of them

showed resistance to a single drug and four to more than one first-line drug. All of the single-drug resistant strains were resistant to isoniazid. Of 100 isolates, 56 were resistant to capreomycin, 41 to kanamycin, 12 to para-aminosalicylic acid and four to ethionamide. All of the first-line drug-resistant strains were found to be susceptible to para-aminosalicylic acid and ethionamide.

In view of the above findings, we suggest that clinicians should be well-informed about the current local epidemiology of tuberculosis, and health care institutions should maintain up-to-date drug susceptibility data on the local isolates of *M. tuberculosis*.

Key Words: *Mycobacterium tuberculosis*, isoniazid, rifampin, ethambutol, streptomycin, kanamycin, para-aminosalicylic acid, ethionamide, capreomycin, susceptibility

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Introduction

Tuberculosis (TB) has been spreading worldwide in recent years. More and more singletons seem to indicate that the incidence of drug-resistant *Mycobacterium tuberculosis* is increasing in almost all industrialized and undeveloped countries. One-third of the world population is infected with this pathogen, and eight million new tuberculosis cases occur each year. Moreover, nearly three million people die annually of tuberculosis, making it the leading cause of death due to an infectious agent worldwide (1,2).

TB is a widely spreading infectious disease in Turkey also. Since 1985, there have been no extensive studies on a national level concerning the epidemiologic features of tuberculosis. There is no definite data about the current prevalence in Turkey (3). In an investigation carried out in 80,000 persons, tuberculosis prevalence was found to be 3.58‰ between 1981 and 1982 in Turkey (4). According to this rate, Turkey is in the group of hyperendemic countries. According to the data published

by the Health Ministry Presidency Department of Struggle Against Tuberculosis, tuberculosis incidence increased between 1980 and 1985 and decreased between 1985 and 1992 (5).

The modern era of tuberculosis is characterized by an increase in the number of cases of infections with multiple drug resistant (MDR) *M. tuberculosis*. The rising prevalence of MDR strains has resulted in outbreaks and individual cases that are only marginally treatable and often fatal. MDR tuberculosis (MDRTB) is caused by a strain of *M. tuberculosis* that is resistant to two or more antituberculosis drugs. Many investigators suggest that the strain should be resistant to isoniazid and rifampin to be qualified as MDR (6,7).

The local epidemiology of MDRTB varies throughout the world. The most significant predictor of MDRTB in all previous studies was history of treatment with antituberculosis drugs. Inadequate therapy remains the most common mechanism by which resistant organisms develop in tuberculosis clinics in many parts of the United

States (6). A susceptible strain of *M. tuberculosis* may become resistant to multiple drugs within a matter of months because of circumstances of monotherapy, erratic drug ingestion, omission of one or more of the prescribed agents, suboptimal dosage, poor drug absorption, or insufficient number of active agents in a regimen. In addition, patients with cavitory lesions have a high frequency of resistance, presumably because they harbour greater numbers of mycobacteria (8). Other high-risk populations for drug resistant tuberculosis include human immunodeficiency virus (HIV) infected and acquired immunodeficiency syndrome (AIDS) patients, immunocompromised individuals, socioeconomically indigent individuals and inner-city dwellers, including homeless incarcerated individuals (6).

Mortality from MDRTB exceeds 80% in persons infected with HIV but is also high in patients free of HIV. The management of MDRTB is complicated by the lack of methods for rapid detection of resistant strains of *M. tuberculosis* (7).

The prevalence of drug-resistant organisms among patients with pulmonary tuberculosis in Turkey has steadily increased from 22% to 39% in the past five decades. Inadequate therapy remains the most common mechanism by which resistant organisms develop in tuberculosis clinics in many parts of Turkey (9).

In another article, chosen from 67 articles reviewed by Ucar, it was indicated that in the last 40 years there has been no difference in the rate of resistance to first-line drugs. According to the author, standard drug concentrations were not used in the articles which were included in the investigation (10).

Drug-resistant strains of *M. tuberculosis* can be transmitted by infected individuals or resistance can be acquired during-therapy for drug susceptible diseases. At least until susceptibility test data are available, the recommended initial treatment for tuberculosis consists of isoniazid, rifampin, pyrazinamide, and either ethambutol or streptomycin. Resistance has led to variations in this regimen that sometimes include more toxic alternative drugs including ethionamide, aminosalicylic acid, kanamycin and capreomycin, as well as ciprofloxacin and ofloxacin. The drug regimens used for retreatment usually include alternative drugs. Success in treating drug-resistant tuberculosis varies (8,11). Recent outbreaks of tuberculosis with MDRTB have indicated an urgent need for new drugs.

The aim of this study was to determine the activities of first-line and second-line antituberculosis drugs against 100 strains of *M. tuberculosis* isolated from various clinical specimens.

Materials and Methods

Mycobacterial strains. One hundred strains of *M. tuberculosis* isolated from various clinical samples in the Clinical Microbiology Laboratory of Ege University Hospital were included in this study. All strains were isolated by culturing on Löwenstein-Jensen slants and by use of the MB/BacT (Organon-Teknika) automated system. Organisms were identified to species level by standard methods (12). *M. tuberculosis* ATCC 27294, ATCC 35838, ATCC 35825 and ATCC 35837 were used for internal quality control.

Antimicrobial agents. Standard laboratory powders with known potency were used. Isoniazid (INH), rifampin (RIF), ethambutol (ETB), streptomycin (STR), kanamycin (KM), para-aminosalicylic acid (PAS), ethionamide (ETH) and capreomycin (CAP) were obtained from Sigma. The following drugs and concentrations were included in agar proportion susceptibility tests: INH 0.2 and 1 µg/ml, RIF 1 µg/ml, ETB 5 µg/ml, STR 2 µg/ml, KM 5 µg/ml, PAS 2 µg/ml, ETH 5 µg/ml and CAP 10 µg/ml. Antibacterial activity was determined by an agar dilution technique using Middlebrook's 7H10 agar. Standard Middlebrook 7H10 agar and oleic-albumin-dextrose-catalase (OADC) enrichment were used to prepare all drug-containing media (12). Stock solutions of the agents were prepared on the day of testing according to the recommendations of the manufacturers.

Susceptibility testing. Standard agar proportion dilution methods were used in this study (12). Colonies from a Löwenstein-Jensen tube were homogenized in phosphate buffered saline (pH 7.0) to achieve turbidity equal to a McFarland 1.0 standard, corresponding to approximately 10^9 CFU/ml. This bacterial suspension was used for agar dilution by inoculating plates with a Steers replicator. In the agar proportion dilution methods, an isolate was classified as susceptible to a drug if the number of colonies that grew on the drug-containing plate was < 1% of the number of colonies that grew on a control plate without the drug, partially resistant if the number was between 1 and 10%, and resistant if the number was >10%. In cases where two drug

concentrations were tested in the agar proportion dilution method, an isolate was classified as partially resistant if it exhibited resistance at the lower concentration but was susceptible at the higher of the two concentrations tested.

Results

The antimycobacterial activities of the first- and second-line drugs are summarized in Tables 1 and 2. First-line drug-resistant strains were isolated from 10 (10%) clinical samples. Six of them (6%) were resistant to a single drug and four (4%) were resistant to more than one first-line drug [INH and STR, INH and ETB, ETB and STR, RIF and STR] (Table 2). All single drug-resistant strains were resistant to INH.

Of the second-line drug-resistant strains, 56 were resistant to CAP, 41 to KM, 12 to PAS and four to ETH.

Two of the six isolates which were resistant to INH were susceptible to second-line drugs, three of them were resistant to KM and CAP and one of them was

resistant only to CAP (Table 2). All of the four isolates that were resistant to more than one first-line drug were susceptible to second-line drugs.

All of the first-line drug-resistant strains were found to be susceptible to PAS and ETH.

Discussion

TB incidence is still increasing and the most serious aspect of the problem is the recent outbreaks of MDRTB which pose an urgent public health problem and require rapid intervention. When the infecting organism is resistant to both INH and RIF, the duration of treatment is prolonged from six months to 18-24 months, and the cure rate decreases from nearly 100% to less than or equal to 60%. The selection of drugs available for treating TB is limited, which makes the treatment of drug-resistant cases particularly difficult (13). In patients infected with MDRTB, at least five drugs are needed to protect against additional acquired resistance. For patients with HIV infection or AIDS in their areas, a six-

	Resistant to one first-line drug (n=6)	Resistant to more than one first-line drug (n=4)	Drug sensitive tuberculosis (n=90)	TOTAL
Resistant to second-line drug	CAP 4*	-	52	56
	KM 3	-	38	41
	ETH -	-	4	4
	PAS -	-	12	12

Table 1. Resistance to first- and second line drugs (n = 100).

* Isolates, which were resistant to CAP, include the three KM-resistant isolates.

Samples	Resistant to one first-line drug and more than one first-line drug	Resistant to second-line drug
1	INH	KM, CAP
2	INH	KM, CAP
3	INH	KM, CAP
4	INH	CAP
5	INH	-
6	INH	-
7	INH, STR	-
8	INH, ETB	-
9	ETB, STR	-
10	RIF, STR	-

Table 2. Resistance of the first-line drug-resistant strains to second-line drugs.

drug regimen based on the local susceptibility pattern of the patient-infecting organisms is defined (8).

The aim of the present study was to determine the activity of first- and second-line antituberculosis drugs on clinical isolates of *M. tuberculosis*. The rate of susceptibility to first-line drugs varies in different parts of the world, including Turkey. Studies on the rates of the resistance to first-line drugs which were carried out in different areas of Turkey are summarized in Table 3. In the reports in which standard drug concentrations were used the number of strains studied varied between 50 and 393. The total rate of resistance varies between 14.3% and 33.7%, which resists at least one first-line drug (total resistance) and the rate of MDRTB varies between 6% and 56.2% (14-24). When the rates of resistance were inspected year by year between 1987 and 1999, it was seen that the rate of resistance to either of the first-line drugs increased from 19.2% in 1987 to 22.7% in 1991. When the rates of resistance to drugs were evaluated one by one between 1987 and 1991, it was seen that the resistance to INH changed from 2.9%

to 14.3%, RIF from 5.8% to 12%, ETB from 2.2% to 8.5 and STR from 2.4% to 11.4% (16). In Sureyyapasa Chest Disease Hospital, the rate of resistance to either of the first-line drugs was determined to be 11.2% in 1980, 14.5% in 1986, 18% in 1987 and 17.4% in 1988 (25). According to Goral et al. (23), the total rate of resistance to first-line drugs increased from 31% in 1982-1985 to 45.7% in 1985-1987. In an analysis by Ucar, 67 articles in which resistances of first-line and second-line drugs were reviewed, primary rate resistances to first line drugs were determined to be 9% in STR, 4% in RIF, 5% in INH and 0.4% in ETB and primary total rates of resistance were determined to be 16%, 11%, 13% and 3%, respectively. It was concluded that there has been no statistically significant difference in the rates of drug resistance over the last 40 years. Rates of secondary resistance are given as 9% in STR, 7% in RIF, 14% in INH and 0% in ETB. Secondary total rates of resistances are given 30%, 25%, 35% and 13%, respectively. The resistance rate against another drug was indicated as 45%, against two drugs as 21%, and 13% against three

Table 3. Resistance to first-line drugs in various region of Turkey.

City-Year (Reference)	Number of strains	Methods	Drug concentration in medium				Resistant to first-line drugs (%)				Primary-Secondary resistant/ Total resistant (%)	MDRTB (%)
			INH	RIF	STR	ETB	INH	RIF	STR	ETB		
SAMSUN 1998 (14)	50	Proportion ¹	0.2-1	1.0	2.0	2.0-10	6	4	2	8	- / 20	6%
ANKARA 2000 (15)	100	Proportion ¹	0.2	1.0	2.0	5.0	19	18	9	13	- / 27	12
		BACTEC	0.2	2.0	6.0	7.5	19	17	10	13		
SIVAS 1987-1991 (16)	226	Proportion ²	-	-	-	-	10.2	8	8.5	4.9	- / 14.3-22.7	-
GAZIANTEP 1994-1998 (17)	199	BACTEC	0.2	2.0	6.0	7.5	10.6	0.5	2	4.5	- / 17.6	15.6 (two drugs) 12 (three drugs)
ISTANBUL 1995-1997 (18)	32	Proportion ²	0.2-1	20-40	4-8	2-3	100	100	22	15	Secondary resistant to 32 strains	56.26 (two drugs) 15.6 (three drugs)
ADANA 1993-1995 (19)	393	BACTEC	0.2	2.0	6.0	7.5	5.08	3.82	4.58	1.01	- / 32.31	17.81
EDIRNE 1996 (20)	70	Proportion ¹	0.2-1	1	2-10	5	30 P	11 P	32 P	9 P	52-62 / INH 30 - RIF 11 - STR 39 - ETB 13	7
							31 P	12 P	50 P	19 P		
IZMIR 1986-1988 (21)	59	Proportion ²	0.2	20	4	2	6.77	5.08	10.1	0	- / 22.01	20.33
IZMIR 1989-1992 (22)	63	Proportion ²	0.2	20	4	2	9	27	32	3	41 (total primary resistant)	22.5
BURSA 1990 (23)	193	Proportion ²	0.2-1	20-40	4-8	5-10	23.8	6.7	16.6	4.1	- / 33.7	20 (two drugs) 9.2 (three drugs)
MANISA 2000 (24)	75	Proportion ¹	1.0	1.0	2.0	6.0	16	12	20	13	- / 25	6 (two drugs) 2 (three drugs)

¹ Middlebroke 7H10 +OADC medium

² Löwenstein/Jensen medium

P Primary resistant

S Secondary resistant

drugs (10). Our results show that of 100 *M. tuberculosis* strains, 10 (10%) were resistant to at least one of the first-line antituberculosis drugs. Six (6%) of these were resistant to one drug (INH), while four (4%) were resistant to more than one. Our total resistance rates are lower than the general resistance rates found so far in Turkey. This difference can be attributed to several features of the patient population. Our patients were from a relatively higher socioeconomic level and were receiving directly observed therapy (DOT). In contrast, previous studies in Turkey included socioeconomically indigent outpatients who were admitted to State Tuberculosis Dispensaries and the compliance of the patients was questionable.

The drug resistance of tubercle bacilli has consequently led to variations in therapy regimens that sometimes include more toxic alternative drugs including ethionamide, para aminosalicylic acid, kanamycin and capreomycin. The rate of susceptibility to second-line drugs varies in different parts of the world, including Turkey. There are few articles examining the resistance to second-line drugs in Turkey. In research on 32 MDRTB strains, the resistance to ETH was found to be 65.6% (20 µg/ml) and 56.2% (40 µg/ml), to KN 9.3% (20-30 µg/ml), and to CAP 12.5% (20 µg/ml) and 6.2% (40 µg/ml) (18). In Ucar's analysis, five studies were examined and primary resistance to PAS between 1963 and 1983 was found to increase from 3.6 to 8%. Secondary resistance to PAS between 1963 and 1984 was found to increase from 6 to 15%. Primary rates of resistance to ETB between 1990 and 1993 were found to be 0-11% and secondary resistance rates were 7-28% in the same years (10). In previous studies, KM resistance was found to be 1.1% in Thailand, 25% in India and 6% in Mexico, and in the Russian Federation there is an increase of 3-6% annually. The resistance in ETH was found to be 65.1% in India, 7% in Mexico and 1.7% in Italy and all MDRTB cases in Ethiopia were sensitive to ETB. In Mexico the resistance to PAS was 9% while in England resistance to CAP was 0.3% (26-31). The levels of resistance to second-line drugs were 41% for KM, 56% for CAP, 4% for ETB and 12% for PAS in this study. Multidrug-resistant isolates were susceptible to all second-line drugs. All isolates that were resistant to first-line drugs were susceptible to PAS and ethionamide.

Isolates resistant to KM were also resistant to CAP. The resistance rates to KM and CAP were higher. This may be the result of extended use of aminoglycosides such as amikacin and gentamycin for the empirical treatment of lower respiratory tract infections. The early period of tuberculosis can be misdiagnosed as a nonspecific infection of the lower respiratory tract, and thus the difference may be due to the irrational unlimited use of aminoglycosides in the treatment of nonspecific infections. *M. tuberculosis* can develop resistance to KM as a result of cross-resistance between the aminoglycosides. This situation may explain why the KM resistance rate is high among our isolates. Significantly, there is no cross-resistance between KM and STR. However, cross-resistance between KM and CAP can occur. This may be an explanation for the lower resistance rate of STR found in this study. This may also explain why the resistance rates of both KM and CAP are high. These findings show that ETB and PAS can be good alternatives, particularly in MDRTB cases.

Most countries affected by the HIV pandemic and increasing prevalence of tuberculosis also have poorly functioning tuberculosis control programmes and cannot afford the antituberculosis drug regimens that are most effective for preventing MDR disease as well as treating it. Thus, the stage is being set for a substantial increase in the incidence of drug-resistant tuberculosis in many countries. If this is allowed to occur, the developing countries will not be the only ones affected. International travel, migration between countries and trade with emerging economies continue to increase, and thus the proportion of tuberculosis cases in developed countries that originate from developing countries will increase as well (32). In the light of these findings, two suggestions can be put forward. First, clinicians should know the local epidemiology of tuberculosis. Second, health care institutions (hospitals and public health departments) should maintain up-to-date drug susceptibility data on the local isolates of *M. tuberculosis*.

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