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Primary Drug Resistance and Molecular Epidemiology of the *Mycobacterium tuberculosis* Strains Isolated in the Kelkit Valley*

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Aim: The aim of this study was to determine the primary drug resistance rates and genotypes of resistant *Mycobacterium tuberculosis* strains isolated in the Kelkit Valley.

Materials and Methods: Primary resistance to isoniazid (INH), rifampicin (RIF), ethambutol (ETB), and streptomycin (SM) was determined with the BACTEC 460 radiometric system. Forty-eight resistant *M. tuberculosis* strains isolated from tuberculosis patients living in the Kelkit Valley were genotyped using the spoligotyping method.

Results: Approximately 11.6% of the strains were resistant against at least one major drug, and 8.3% were resistant against more than one drug. Single-drug resistance for INH, ETB, SM, and RIF was found as 5.8%, 2.1%, 3.7%, and 0.0%, respectively. Two or more drug resistance rates for SM + ETB, SM + INH, INH + RIF, RIF + ETB, INH + ETB + RIF, SM + ETB + INH and INH + ETB + RIF + SM were determined as 0.8%, 1.7%, 0.4%, 0.8%, 2.9%, 0.4%, and 1.2%, respectively. Fifteen spoligotype groups were created when the results were compared with the world databank (SpoIDB4). Families of four strains could not be determined. The spoligotype groups most frequently encountered in our study were ST4 (n = 10, 20.8%), ST53 (n = 6, 12.5%), ST7 (n = 4, 8.3%), ST41 (n = 4, 8.3%) and ST31 (n = 4, 8.3%), and the most frequently encountered families were TI (n = 18, 37.5%), S (n = 12, 25%) and LAM-7 TUR (n = 4, 8.3%).

Conclusions: High rates of primary resistance against major anti-tuberculosis drugs, the LAM-7 TUR clone, and high grouping rates were detected in this study, the first such study carried out in the Kelkit Valley.

Key Words: *M. tuberculosis*, primary drug resistance, molecular epidemiology

Kelkit Vadisinden İzole Edilen *Mycobacterium tuberculosis* Suşlarında Primer İlaç Direnci ve Moleküler Epidemiyoloji

Amaç: Bu çalışmada; Kelkit vadisinden izole edilen *Mycobacterium tuberculosis* izolatlarında primer ilaç direncinin ve direnç genotiplerinin belirlenmesi amaçlanmıştır.

Yöntem ve Gereç: İzolatların, izoniazid, rifampisin, etambutol ve streptomisin dirençleri BACTEC metoduyla belirlendi. Kırk sekiz dirençli *Mycobacterium tuberculosis* suşu spoligotiplendirme yöntemiyle genotiplendirildi.

Bulgular: En az bir ilaca direnç % 11.6, birden fazla ilaca direnç % 8.25 olarak belirlendi. Tek ilaç direnci sırası ile izoniazid (INH), etambutol (ETB), streptomisin (SM) ve rifampisin (RIF) için % 5.8, % 2.07, % 3.73 ve % 0 bulundu. İki veya daha fazla ilaca direnç oranları; streptomisin + etambutol, streptomisin + izoniazid, izoniazid + rifampisin, rifampisin + etambutol, izoniazid + etambutol + rifampisin, streptomisin + etambutol + izoniazid ve izoniazid + etambutol + rifampisin + streptomisin için sırasıyla; % 0.82, % 1.65, % 0.41, % 0.82, % 2.90, % 0.41 ve % 1.24 olarak belirlendi. Spoligotiplendirme sonuçlarına göre 15 farklı genotip paterni elde edildi. Bu yöntemle 4 izolat tiplendirilemedi. Çalışmamızda en sık rastlanan spoligotip kümeleri sırası ile ST4 (n = 10, % 20.8), ST53 (n = 6, % 12.5), ST7 (n = 4, % 8.3), ST41 (n = 4, % 8.3), ST31 (n = 4, % 8.3); ve familyaları TI (n = 18, % 37.5), S (n = 12, % 25) ve LAM-7 TUR (n = 4, % 8.3) olarak saptandı.

Sonuç: Kelkit vadisinde yapılan bu ilk çalışmada; major anti tüberküloz ilaçlara karşı yüksek oranda direnç varlığı gözlendi.

Anahtar Sözcükler: *M. tuberculosis*, primer ilaç direnci, moleküler epidemiyoloji

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Introduction

Tuberculosis remains one of the major public health problem worldwide, despite modern diagnostic techniques and appropriate treatment regimens. The World Health Organization (WHO) has reported that one-third of the world's population is infected with tuberculosis bacilli and approximately two million people die from tuberculosis every year (1). In Turkey, the incidence of tuberculosis was reported as 26/100,000 in 2007 (2). Since drug susceptibility testing can not be performed by all laboratories routinely, antimicrobial chemotherapy is usually given empirically in our country. It is well known that inadequate treatment regimens and incomplete treatments may lead to development of multidrug-resistant strains. It is also reported that treatment and care of multiple-drug-resistant patients is rather difficult, and the mortality is high (1). The determination of resistance rates and knowledge about the development and spread of resistant isolates may provide a valuable baseline for the establishment of control strategies against tuberculosis. Many studies have been carried out in Turkey on the resistance rates and genotyping of *Mycobacterium tuberculosis* (1,3-10). However, the Kelkit Valley was not included in these surveys. The Kelkit Valley is a transitional zone geographically between Central Anatolia and the Middle and East Black Sea regions. There is no migration to this area from other parts of the country. In this study, we aimed to determine the rate of primary drug resistance and genotypes of resistant *M. tuberculosis* strains isolated in this area.

Materials and Methods

Study Population

Forty-eight resistant tuberculosis strains isolated from 300 acid-resistant bacilli (ARB)-positive sputum samples in the Kelkit Valley between December 2004 – February 2007 were included in this study (Table 1). Ages of the subjects ranged from 5 to 78 (≤40: 180 persons, ≥40: 61 persons); 90 (37.4%) were female and 151 (62.6%) male.

Of the patients having resistant strains, 31 were male, 17 were female, 36 were under the age of 40, 12 were over the age of 40, at least one person had tuberculosis in the families of 8 patients, friends of 3 patients had tuberculosis, 40 were born in the Kelkit Valley, 22 were smokers, 19 had undergone BCG vaccination, 9 used alcohol, and 36 had low socioeconomic status (monthly income <200 YTL). They were diagnosed for the first time and had not used any antituberculosis drugs before. The location of the Kelkit Valley is shown in Figure 1.

Table 1. Drug resistance rates of the 241 *M. tuberculosis* strains.

Drugs	Number of resistant strains (%)
INH	14 (5.8%)
ETB	5 (2.1%)
SM	9 (3.7%)
RIF	0 (0.0%)
SM + ETB	2 (0.8%)
SM + INH	4 (1.7%)
INH + RIF	1 (0.4%)
RIF + ETB	2 (0.8%)
INH + ETB + RIF	7 (2.9%)
SM + ETB + INH	1 (0.4%)
INH + ETB + RIF + SM	3 (1.2%)
Total	48 (19.9%)

INH: Isoniazid. SM: Streptomycin. ETB: Ethambutol. RIF: Rifampicin.

Culture-Antibiotic Sensitivity Testing

All sputum specimens were digested and decontaminated by the N-acetyl-L-cysteine sodium hydroxide (NALC – NaOH) method as defined by the Centers for Disease Control (CDC) (11) and then inoculated into BACTEC 12B bottles (Becton Dickinson, Cockeysville, MD, USA). BACTEC bottles were incubated at 35°C and read by using a BACTEC Model 460 TB radiometric system (Becton Dickinson Diagnostic Instruments, Sparks, MD, USA) every day for 6 weeks. BACTEC 12B bottles with growth index values of ≥100 were sampled for Ziehl Neelsen stain. *M. tuberculosis* was identified in BACTEC using the NAP (r-nitro- -acetylamino- -hydroxy-propiophenone) test, which differentiates it from mycobacteria other than tuberculosis (MOTT).

Isoniazid (INH), streptomycin (SM), rifampicin (RIF) and ethambutol (ETB) were obtained from Sigma Chemical (USA). Antibiotic sensitivity tests for the *M. tuberculosis* complex isolates were carried out in the

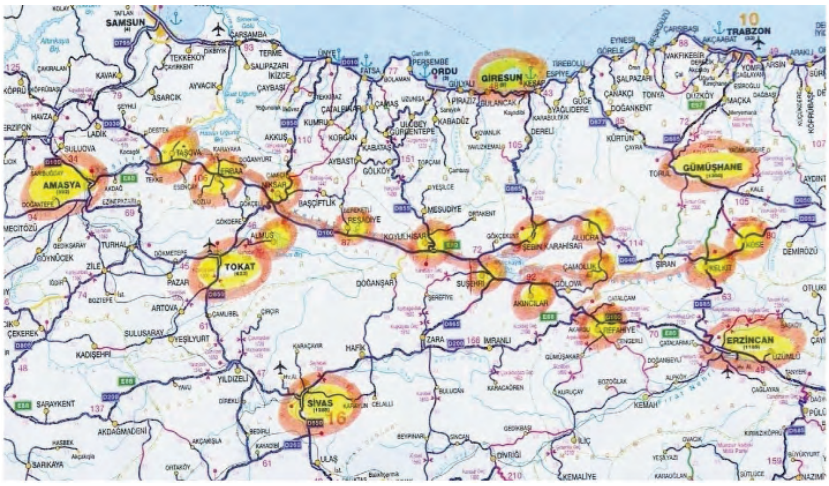

Figure 1. Regions of the Kelkit Valley where the clinical isolates were obtained.

BACTEC 460 TB radiometric system (12). The standard *M. tuberculosis* H37Rv American Type Culture Collection (ATCC) 27294 strain was used for quality control of the NAP and antibacterial sensitivity tests.

Spoligotyping

Spoligotyping was carried out with the method previously used by Kamerbeek et al. (13). The whole direct repeat (DR) region of *M. tuberculosis* genome was amplified by polymerase chain reaction (PCR) with Dra and Drb primers, with primer Dra biotinylated at the 5' end. *M. tuberculosis* H37Rv and *M. bovis* BCG strains were used as positive controls. The genomic DNA of the mycobacteria was extracted from cultured cells as described by Kolk et al. (14). 25 µl of reaction mixture was prepared for PCR with 12.5 µl HotStarTaq Master mixture (Qiagen, Hilden, Germany; 1.5 mM $MgCl_2$ and 200 µM deoxynucleotide triphosphate), 2 µl primers (each 20 pmol), 5 µl DNA solution (ca. 10 ng), and 3.5 µl distilled water. The mixture was heated for 15 min at 96°C and amplified in 30 cycles as 1 min in 96°C, 1 min in 95°C, and 30 sec in 72°C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one unique spacer DNA sequences within the DR locus. After the hybridization, the membrane was washed in 2 X SSPE for 10 min [1X SSPE: 0.8 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA (pH 7.7)].

The product was kept in 0.5% sodium dodecyl sulfate for 60 min, and then incubated with 1/4000 diluted conjugate in 42 °C for 60 min. The membrane was washed in 2 X SSPE for 10 min for the second time and then kept in 0.5% sodium dodecyl sulfate for 42 min and slightly shaken with 2 X SSPE at room temperature for 5 min afterwards. Hybridized DNA was detected with the chemiluminescence method (Amersham Corp., Arlington Heights, IL, USA) according to the manufacturer's instructions, and X-ray films (Hyperfilm ECL; Amersham, Biosciences, Bucks, United Kingdom) were obtained (15,16).

The chi-square test, Spearman's rank order correlation coefficient, and exact chi-square test were used for the statistical comparisons.

Results

The study represents 28.7% of the patients who were newly diagnosed as tuberculosis in the last three years in the Kelkit Valley (300/1045 ARB-positive patients).

Drug Resistance

Of the 241 *M. tuberculosis* strains, 193 (80%) were resistant against all tested drugs, 28 (11.6%) were resistant against at least one drug, and 20 (8.3%) were resistant against more than one drug (Table 1).

Resistance status according to the different demographic distributions, such as place of birth, alcohol consumption, smoking, and presence of a BCG vaccine,

are shown in Table 2. All patients included in this study live inside the Kelkit Valley. Although their birth places differ, their hometown is Kelkit Valley, and they have lived in this region for at least five years.

Spoligotyping

Fifteen groups were created; family names of four strains could not be determined when the spoligotyping results of the 48 resistant strains were compared with the world databank (SpoIDB4) (Table 3).

The most frequently encountered spoligotype groups in our study were: ST4 (n = 10, 20.8%), ST53 (n = 6, 12.5%), ST7 (n = 4, 8.3%), ST41 (n = 4, 8.3%), ST31 (n = 4, 8.3%), and 2 strains (n = 2, 4.1%) each of ST1564, ST803, ST34, ST50, ST196, ST262, ST36, ST218, ST284 and ST2067. The most frequently encountered spoligotype families were: TI (18, 37.5%), S (12, 25%), HI (4, 8.3%), LAM-7 TUR (4, 8.3%), H3 (2, 4.1%), H4 (2, 4.1%), H3-H4 (2, 4.1%), and xI (2, 4.1%).

Discussion

The emergence of drug-resistant tuberculosis has been documented worldwide (1,17). Drug resistance in tuberculosis may be classified as primary or secondary. Primary drug resistance is defined as resistance to anti-tuberculosis drugs in a patient who never received therapy (17) while secondary resistance occurs during anti-tuberculosis therapy (18). Resistance to more than one anti-tuberculosis drug is referred to as multiple-drug resistance (17).

Several studies have been conducted in Turkey about primary drug resistance rates. The most comprehensive study was a meta-analysis study comparing the periods between 1984-1989 and between 1990-1995 that was carried out by Yolsal et al. (19) (Table 4). They concluded that primary resistance to INH was decreased in the second period, while resistances to ETB, SM and RIF were increased. Bengisun et al. (4) reported a study from 1976-1997, in which they found that total resistances to INH, RIF and SM were 10.5%, 6.9%, and 7.0%, respectively. In 2002, Kiliçaslan et al. (6) detected that

Table 2. Distribution of drug resistance rates by demographic and clinical variables.

Variables	Sensitive (%)	Resistant (%)	Total	P
Gender				
Female	73 (30.3%)	17 (7.1%)	90	$x^2 = 0.10$
Male	120 (49.7%)	31 (12.9%)	151	P = 0.757
Age				
≤ 40	144 (59.7%)	36 (14.9%)	180	$x^2 = 0.00$
≥ 41	49 (0.3%)	12 (5.1%)	61	P = 0.956
Birthplace				
Kelkit Valley	154 (64%)	40 (16.6%)	194	$x^2 = 0.00$
Outside Kelkit Valley	39 (16%)	8 (3.4%)	47	P = 0.986
Alcohol				
Uses	33 (13.7%)	9 (3.7%)	42	$x^2 = 0.00$
Abstains	160 (66.3%)	39(16.3%)	199	P = 0.954
Smoker				
Yes	86 (35.7%)	22 (9.2%)	108	$x^2 = 0.00$
No	107 (44.3%)	26 (10.8%)	133	P = 0.99
BCG Vaccine				
Yes	78 (32.3%)	19 (7.9%)	97	$x^2 = 0.00$
No	115 (47.7%)	29 (12.1%)	144	P = 0.952

Table 3. The group and family distribution of the strains following comparison with the world databank (SpoIDB4).

No	Octal value	Binary value	ST	CLADE	R
1	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	INH
2	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	SM
3	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	INH + ETB + SM
4	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	RIF + ETB
5	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	INH
6	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	INH
7	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	SM
8	7763777777760771	nnnnnnnnnoonnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	34	S	INH
9	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	SM
10	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	INH
11	0000000007760771	ooooooooooooooooooooooonnnnnnnnnnooooonnnnnnn	4	S	INH + ETB + RIF + SM
12	7763777777760771	nnnnnnnnnoonnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	34	S	INH + ETB + RIF
13	7777777777760771	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	53	T1	INH + ETB + RIF + SM
14	7777777777760771	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	53	T1	INH + ETB + RIF
15	7777777777760771	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	53	T1	SM
16	7777777777760771	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	53	T1	ETB
17	7777777777760771	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	53	T1	INH
18	7777777777760771	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	53	T1	RIF + ETB
19	7740377777760700	nnnnnnnnooooooonnnnnnnnnnnnnnnnnnnnnnooooonnnoooo	31	T1	ETB
20	7740377777760700	nnnnnnnnooooooonnnnnnnnnnnnnnnnnnnnnnooooonnnoooo	31	T1	INH + ETB + RIF
21	7777400077760771	nnnnnnnnnnnnnnooooooooooonnnnnnnnnnooooonnnnnnn	803	T1	SM
22	7777400077760771	nnnnnnnnnnnnnnooooooooooonnnnnnnnnnooooonnnnnnn	803	T1	INH + ETB + RIF
23	3777777777760771	onnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	7	T1	SM + ETB
24	3777777777760771	onnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	7	T1	INH
25	3777777777760771	onnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	7	T1	SM
26	3777777777760771	onnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	7	T1	SM
27	6777777777760771	nnonnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	196	T1	INH
28	6777777777760771	nnonnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	196	T1	ETB
29	7740377777760700	nnnnnnnnooooooonnnnnnnnnnnnnnnnnnnnnnooooonnnoooo	31	T1	SM + ETB
30	7740377777760700	nnnnnnnnooooooonnnnnnnnnnnnnnnnnnnnnnooooonnnoooo	31	T1	INH + SM
31	7777774047607 71	nnnnnnnnnnnnnnnnnnnnnooooooonoonnnnnnooooonnnnnnn	41	LAM-7 TUR	INH
32	7777774047607 71	nnnnnnnnnnnnnnnnnnnnnooooooonoonnnnnnooooonnnnnnn	41	LAM-7 TUR	INH + RIF
33	7777774047607 71	nnnnnnnnnnnnnnnnnnnnnooooooonoonnnnnnooooonnnnnnn	41	LAM-7 TUR	ETB
34	7777774047607 71	nnnnnnnnnnnnnnnnnnnnnooooooonoonnnnnnooooonnnnnnn	41	LAM-7 TUR	SM
35	7747777774207 71	nnnnnnnnoonnnnnnnnnnnnnnnnnnnnnnnnooonooooonnnnnnn	262	H4	INH + ETB + RIF
36	7747777774207 71	nnnnnnnnoonnnnnnnnnnnnnnnnnnnnnnnnooonooooonnnnnnn	262	H4	INH + SM
37	7777377777207 71	nnnnnnnnnnnnnonnnnnnnnnnnnnnnnnnnnonooooonnnnnnn	36	H3-T3	INH + ETB + RIF
38	7777377777207 71	nnnnnnnnnnnnnonnnnnnnnnnnnnnnnnnnnonooooonnnnnnn	36	H3-T3	INH + SM
39	7777767377605 71	nnnnnnnnnnnnnnnnnnnonnnonnnnnnnnnnnnooooonnnnnn	1564	x1	INH
40	7777767377605 71	nnnnnnnnnnnnnnnnnnnonnnonnnnnnnnnnnnooooonnnnnn	1564	x1	ETB
41	7777777777207 71	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnonooooonnnnnnn	50	H3	INH
42	7777777777207 71	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnonooooonnnnnnn	50	H3	INH + SM
43	7777377740207 71	nnnnnnnnnnnnnonnnnnnnnnnnnnnooooooonooooonnnnnn	218	HI	INH
44	7777377740207 71	nnnnnnnnnnnnnonnnnnnnnnnnnnnooooooonooooonnnnnnn	218	H1	SM
45	0367377777607 71	ooooonnnnnnnoonnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	284	Unknown	INH + ETB + RIF + SM
46	0367377777607 71	ooooonnnnnnnoonnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	284	unknown	INH + ETB + SM
47	7777717777607 71	nnnnnnnnnnnnnnnnoonnnnnnnnnnnnnnnnnnooooonnnnnnn	2067	unknown	INH
48	7777717777607 71	nnnnnnnnnnnnnnnnoonnnnnnnnnnnnnnnnnnooooonnnnnnn	2067	unknown	INH

Table 4. 1984-1989 and 1990-1995 meta analysis results.

Drug	Primary resistance (%) (1984-1989)	Primary resistance (%) (1990-1995)
INH	14.4	8.8
SM	8.8	10.1
ETB	2.2	3.0
RIF	5.7	8.9

INH: Isoniazid. SM: Streptomycin. ETB: Ethambutol. RIF: Rifampicin.

the resistance rate among new cases was 19.9%. In 2003, Durmaz et al. demonstrated that resistance to at least one drug was 33% in Turkey (1). Another study from Istanbul, Turkey, presented in 2007, showed primary drug resistance as 10.9% (5).

The WHO/IUATLD (International Union Against Tuberculosis and Lung Disease) third global report provides data from drug resistance surveys conducted in 76 countries or geographical settings between 1999-2002. The prevalence of any resistance to drugs ranged from 0% (Andorra, Iceland, and Malta)-57% (Kazakhstan). The median prevalence of multidrug resistance in new patients was reported as 1.1%. Multidrug- resistance prevalence in new patients was found to be as high as 14% (Kazakhstan) and 12% (Estonia). On the other hand, no multidrug resistance was observed in Oman, Andorra, Iceland, Lithuania, Luxemburg, Slovenia, Switzerland, Cambodia and New Zealand. The median prevalence rates of resistance to SM, INH, RIF and ETB were found to be 6.3%, 5.9%, 1.4% and 0.8%, respectively (20)

In our study, resistance to any drug was found in 28 (11.6%) strains, and resistance to more than one drug was found in 20 (8.2%) strains. We also observed that primary resistance to INH, ETB, SM and RIF was 5.8%, 2.1%, 3.7% and 0%, respectively. Although the frequency of primary resistance to INH was high, primary resistance to INH and RIF together (multidrug resistance) was found in 0.4% of the strains. No statistically significant difference was observed between the drug resistance rates according to the different demographic and clinical variants (Table 2).

High primary drug resistance rates indicate ineffective national control programs. It is well established that the rates of drug resistance vary from country to country and from region to region even in the same country. These variations are associated with demographic characteristics of the patients, inadequate treatment protocols, availability of anti-tuberculosis drugs without prescription and the changes in drug concentration (21,22). Like other parts of our country, the high resistance rates that we detected in the Kelkit Valley indicate the active transition of the drug-resistant strains and the ineffective tuberculosis control programs in our region. It is thus essential to regularly screen surveillance of drug susceptibility patterns for administration of effective treatment and control programs.

When the spoligotyping results of the 48 resistant strains were compared with the world databank (SpoIDB4), the most frequently encountered spoligotype groups, ST4 and ST53, were seen to be consistent with the common types in the world. A previous study from Turkey has shown that ST41, ST53 and ST50 were the most common spoligotype patterns in Turkey (22). Another study conducted in different regions of Turkey showed that the major spoligotyping-defined –shared –types were ST41 (22.5%), ST53 (19.5%), ST50 (6.5%), ST1261 (4.5%) and ST47 (3.5%) (7). However, a study from the Military Medical Academy in Ankara documented that only 7.9% of isolates belonged to the ST41 spoligotypes. They have also reported that Beijing genotype was found in only two strains (9). In our study, the most frequent spoligotype families were TI (n = 18, 37.5%) and S (n = 12, 25%), and these spoligotype families are encountered in different regions of the world (23-25). We also found some isolates belonging to the LAM-7 TUR (n = 4, 8.3%) family. This family was detected by Durmaz et al. (1) in Malatya, a region close to ours, and was considered to be specific to our country. The strain belonging to the Beijing/W family that is encountered throughout the world, especially in the Asian countries, has not been observed, although Koksalan et al. (8) reported that the prevalence rate of the Beijing genotype was 1.1% in Istanbul. Another study conducted in Izmir, including 56 *M. tuberculosis* strains, documented only one isolate as belonging to the Beijing family (10). Similarity between the 15 different spoligotype groups detected in our region and the national and international spoligotype groups indicates a heterogeneous *M. tuberculosis* population. This is the first study that has documented information regarding the primary drug resistance and distribution of *M. tuberculosis* genotypes in the Kelkit Valley.

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