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Molecular epidemiology of tuberculosis cases admitted to Ege University Medical Faculty Hospital

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Abstract:

BACKGROUND AND AIM: Advances in molecular diagnostic tools for tuberculosis (TB) have improved the typing of TB. Leading to the identification of infection origins in populations and early detection of resistant TB strains. In this study, we used molecular techniques to identify, genotype, and determine the drug resistance of TB bacilli in samples from patients with pulmonary and extra-pulmonary TB at Ege University Hospital. Data were analyzed for correlations with biochemistry, clinical, and radiological aspects of the disease.

METHODS: Molecular typing was performed using the Spacer Oligonucleotide Typing (spoligotyping) method on samples from 402 patients diagnosed with pulmonary and extra-pulmonary TB at Ege University Hospital between 2009 and 2014. We retrospectively extracted demographic data from patient files, including clinical and radiological findings, case origin, diagnosis date, comorbidities, treatment, drug-resistance patterns, biochemistry, tuberculin skin test, interferon-gamma release test, length of hospital stay, and one-year mortality.

RESULTS: The study included 402 patients (238 males/164 females, mean age: 52±18.4 years) with a microbiological diagnosis of TB. The patients predominantly originated from the Aegean province (203; 50.5%). Comorbidities were present in 146 (36.3%) patients, with 85 (21.1%) in an immunosuppressed state. The bacilli family was not identified in 52 (12.9%) patients' samples. The most frequently identified family was T1 (40%), followed by H3 (8.2%), LAM7-TUR (7%), H1 (5%), LAM3-S (complex) (4%), and U family (4%). No correlation was found between bacilli families in terms of age, radiological and clinical data, and laboratory findings. Drug resistance patterns were investigated within the families, with the Beijing family showing 55.6% resistance to Rifampicin (R), Isoniazid (H) (1 mg/mL and 0.2 mg/mL), and Streptomycin (S). Hospitalization occurred in 71.6% of patients, and one-year mortality was 17.6%. No differences in hospitalization and mortality rates were found between the families.

CONCLUSIONS: This study revealed that the T1 family is dominant in our community, with a higher percentage of resistant bacilli found in the Beijing family. Further studies on the clinical aspects of these families and those from other areas are necessary to better understand the behavior of TB in our community.

Keywords:

Molecular epidemiology, public health, respiratory infections, tuberculosis

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Introduction

Tuberculosis (TB) is a major global health issue, responsible for over 3 million deaths annually. Countries can be categorized as having low-to-intermediate or high prevalence based on the number of new cases each year. The extensive movement of populations between countries and continents has transformed tuberculosis into a worldwide concern.[1,2] The incidence of TB is gradually increasing, driven by the ease of transmission of *Mycobacterium tuberculosis* and the rising number of immunocompromised patients. Consequently, the epidemiology of tuberculosis has gained further importance in recent years.[3]

Investigating the origin of TB in regions experiencing mass population movements can provide valuable data for global TB control. Several methods are used for the genetic classification of TB strains. In the 1990s, the Insertion Sequence 6110-Restriction Fragment Length Polymorphism (IS6110-RFLP) method was developed, followed by Polymerase Chain Reaction (PCR) methods such as 24-locus Mycobacterial Interspersed Repetitive Units-Variable Number Tandem Repeat (MIRU-VNTR) and Spacer Oligonucleotide Typing (spoligotyping).[4]

Spoligotyping involves analyzing polymorphism in the chromosomal direct repeat (DR) region. Different spacers between DRs are amplified using primers targeting the DR region. The amplification product is then hybridized with 43 different oligonucleotide probes bound to a membrane and complementary to various spacer regions located between the DRs. The presence or absence of these spacer sequences is digitally observed, enabling the distinction of similar or different strains through their spoligotyping patterns. Spacer sequences vary between strains and appear as spots on the fixation surface of the hybridization membrane.[5] It is a simple, fast, and highly reproducible method. However, the discriminatory power of spoligotyping is lower than that of IS6110 typing when analyzing high copy number strains but superior when analyzing low copy strains. The approach can distinguish between *M. tuberculosis* and *M. bovis*.[6]

These methods are recommended and used by the US Centers for Disease Control and Prevention's (CDC) National Tuberculosis Genotyping Service for monitoring TB transmission and control. The United States, with its strict immigration policy, has the ability to control the spread of tuberculosis from highly endemic areas. In contrast, the mass uncontrolled migration seen in many countries, especially in Eastern European and Mediterranean nations, leads to new outbreaks of TB with different genetic characteristics.

The present study presents the findings of a molecular typing study of the TB strains in our region to explore the clinical, radiological, and microbiological differences between alleles. Our country is unique in its representation of a mix of European and Middle Eastern ethnicities, for which there is, as yet, only limited data on genotyping.

Materials and Methods

This retrospective study was conducted with 402 bacteriologically confirmed TB patients diagnosed between 2009 and 2014 in Türkiye. Both pulmonary and extrapulmonary TB patients were included in the study. Materials such as sputum, bronchoscopic aspiration (BASP), bronchoalveolar lavage (BAL), mini-BAL, pleural fluid, peritoneal fluid, pericardial fluid, cerebrospinal fluid (CSF), bone marrow, abscess drainage, urine, and biopsies, sent from various departments of our hospital with a preliminary diagnosis of TB, were first processed in our laboratory through direct staining, as per the current optimal diagnostic approach, and then cultured. The isolates from samples with positive culture results were subjected to identification, genotyping, and drug susceptibility testing.

The study was approved by the Ege University Research Ethics Committee (Approval no: 16-12.1/5 and Approval date: 06/01/2017) and our study was conducted in accordance with the Declaration of Helsinki.

Identification of isolates

The identification of the isolates was performed using the GenoType MTBDRplus (Hain Life Science) test kit, according to the manufacturer's recommendations. Briefly, Deoxyribonucleic Acid (DNA) was isolated from the isolates grown in culture, and the target region was then amplified using biotin-labeled primers. The polymerase chain reaction (PCR) products were hybridized with membrane-bound probes, and the resultant hybrids were visualized as stained bands through the addition of a conjugate and substrate. The resultant banding patterns were evaluated against the evaluation card included in the kit.

Genotyping of isolates

The spoligotyping method was used for genotyping, following the procedure described by the manufacturer of the spoligotyping kit (Spoligotyping Kit: IsogenLifeScience, the Netherlands). Briefly, 5 µL of DNA obtained from the cultured isolates was used as the DNA template for the PCR. Spacers between DR regions were amplified using biotin-labeled primers targeting the DR sequence. The amplified PCR product was hybridized to an oligonucleotide-bound nitrocellulose membrane via a mini blotter. After incubating the membrane with hybridized spacers in a Horseradish Peroxidase (HRP)-conjugate and substrate, we exposed it to X-ray film. This process visualized the hybridized spacers as square-shaped signals. A positive signal indicated the presence of a hybridized spacer, while the absence of spacer hybridization was considered a negative signal. The obtained spoligotypes were compared with strains registered on the SpolDB4 database at http://www.pasteur-guadeloupe.fr and the Mbovis.org spoligotyping database at http://www.mbovis.org/.

Drug susceptibility tests

The susceptibility tests for the isolates were performed using the BACTEC MGIT 960 (BD Spark, USA) automated fluid system. The resistance breakpoints were set at 1.0 µg/mL for rifampicin, 0.1 µg/mL for isoniazid, 1.0 µg/mL for streptomycin, and 5.0 µg/mL for ethambutol.

For all evaluated patients, a retrospective review of patient records was conducted, recording demographic data, place of birth, region, year of TB isolation, history of TB, comorbidities, drug resistance, tuberculosis skin test (TST), contact history, Quantiferon test, clinical and radiological findings, treatments, biochemical data (including leukocytes, neutrophils, lymphocytes, hemoglobin, c-reactive protein (CRP)), hospitalizations, and mortality.

Cases younger than 18 years of age, those with a preliminary diagnosis of tuberculosis but not confirmed by molecular and mycobacteriological methods, those diagnosed at a center other than ours, and those with samples showing mycobacterial growths other than *M. tuberculosis* were excluded from the study.

Statistical analysis

IBM's Statistical Package for the Social Science (SPSS) version 20.0. (Armonk, NY: IBM Corp.) was used for data analysis. Categorical measurements were presented as percentages and numbers, and numerical values as standard deviation (SD) and mean. Categorical values were compared between two groups using the Chi-square test, while numerical values were compared between independent groups using the t-test. Numerical values were evaluated with one-way analysis of variance (ANOVA) for more than two groups. A p-value of <0.05 was considered statistically significant for the entire study.

Results

Our study evaluated samples collected between 2009 and 2014 by Ege University Medical Faculty Mycobacteriology Laboratory from a total of 420 patients diagnosed with TB. After excluding 18 patients under the age of 18 from the study, it was concluded with 402 patients. Of the total 402 (238 males/164 females; mean age: 52±18.4 years) TB patients included in the study, 248 (61.7%) had pulmonary TB, 135 (33.6%) had extrapulmonary TB (EP-TB), and 19 (4.7%) had both (Table 1).

Concerning the characteristics of the diagnosed materials, the culture was positive only in the sputum of 154 (38.3%) patients and only in the BASP sample of 82 (20.4%) patients diagnosed with pulmonary TB. Among those diagnosed with EP-TB, the culture was positive only in the biopsy sample of 69 (17.2%) patients, and only in the pleural fluid sample of 23 (5.7%) patients. The Bacillus Calmette–Guérin (BCG) vaccination status was known for 27 patients, and 22 (5.5%) were vaccinated. During the examination of the diagnosed patients, the Quantiferon test was studied in 26 patients, while 18 had the TST, as indicated in their files. Of the cases, 343 (85.3%) received the standard four-drug therapy.

The molecular typing of *M. tuberculosis* complex (MTC) strains in the laboratory-assessed samples failed to identify the families of 52 (12.9%). A total of 22 different families were identified in all studied samples (Table 2).

The five most commonly identified families were Type 1 (T1) in 40% (63.9% ST53), H3 in 8.2% (69.6% ST50), Latin American and Mediterranean 7 – Türkiye (LAM7-TUR) in 7% (96.4% ST41), H1 in 5% (85% ST47), LAM3 - S (convergent) in 4% (all ST4), and U in 4% (31.2% ST602), respectively. The top ten families and their proportions are also presented in Figure 1. Subtypes with different Sequence Type (ST) numbers were detected within the families, although no statistical assessment could be made as the numbers were not sufficient for a comparison.

Table 1: Demographic characteristics, comorbidities, regions, radiological, and laboratory findings of patients at admission

	n	%
Age (years) (mean±SD)	52±18.4	
Gender		
Female	164	
Male	238	
Region		
Aegean	203	50.5
Mediterranean	17	4.2
Marmara	41	10.2
Central Anatolia	30	7.5
Black Sea	21	5.2
Eastern Anatolia	51	12.7
Southeastern Anatolia	18	4.5
Foreign	20	5
Comorbidities		
No comorbidity	256	63.6
Malignancy	45	11.1
Diabetes	41	10.4
Chronic kidney disease	16	3.9
Other diseases	49	12.1
Immunodeficiency		
Yes	85	21.1
No	315	78.4
Previous TB		
Yes	20	5.0
No	364	90.5
Unknown	18	4.5
TB organ involvement		
Lungs	267	66.4
Lymph nodes	56	13.9
Pleura	32	7.9
Bones–Joints	12	2.9
Other organs	56	13.9
Chest X-Ray findings		
Cavitary lesion	89	22.1
Multilobar involvement	107	26.6
Pleural fluid	59	14.7
Leukocytes (mean [min-max])	7,560/mm^3 (440-26,680/mm^3)	
CRP (mean±SD)	6.6 mg/dL (±7.2)	

SD: Standard deviation, TB: Tuberculosis, CRP: C-reactive protein

When all the families and their numbers were examined, only the top ten groups were included in the assessment due to insufficient data on demographic characteristics, comorbidities, regions, radiological, and laboratory findings for statistical assessment (Table 3, 4).

According to the distribution of family classification by year, the counts were: 2009 in 79, 2010 in 77, 2011 in 69, 2012 in 60, 2013 in 54, and 2014 in 63 samples. When the distribution of years of isolation by families was examined, no statistically significant difference was found (p=0.3) (Table 5).

The distribution of patients by age, region, EP-TB organ involvement, multilobar involvement, and Hemoglobin (Hb) (g/dL) values was heterogeneous, with some differences noted (p=0.02, p=0.04, p=0.03, p=0.05, p=0.04). Consequently, the top ten families were evaluated individually in pairs for these parameters. Only the difference in Hb between the T1 family and the LAM7-TUR family was statistically significant (p=0.042), with no significant differences observed between the other groups. A chest radiograph showed a cavitary lesion in 89 (22.1%) patients, multilobar involvement in 107 (26.1%) patients, and single lobe involvement in 159 (39.6%) patients. In 59 (14.7%) patients, a pleural effusion was detected on chest radiographs. The pleural fluid adenosine deaminase (ADA) was studied in 33 patients, with a median level of 80.1 (8.6–204.4). Radiologically, there was no significant difference in atypical (other than the most commonly involved zones) and typical localizations of the TB families. However, 45 (28%) of the pulmonary TB cases in the T1 family had a typical localization, compared to 13 (46.4%) in the LAM7-TUR family. In the Beijing family, six (66.7%) cases had atypical localization. There was no significant relationship between the pulmonary involvement sites and the distribution by families in those with single lobe involvement.

Of the study's participants, 387 underwent TB treatment. Four patients had unknown treatment status, and 11 patients died before diagnostic clarification, and therefore could not undergo treatment. Regarding drug resistance, there was Rifampicin (R) resistance in 10 (2.5%) patients, Isoniazid (H) at a concentration of 0.2 μg/mL resistance in 41 (10.2%) patients, H at 1 μg/mL resistance in 20 (5%) patients, and Ethambutol (E) resistance in 25 (6.2%) patients. Of the Beijing family, 55.6% showed resistance to R, H (at both 0.2 μg/mL and 1 μg/mL concentrations), and Streptomycin (S), with rates significantly higher than in other families (p<0.001) (Table 6).

Of the patients, 288 (71.6%) were hospitalized due to TB, and 49 died during their hospital stay. The one-year mortality rate was 17.9%. There was no significant difference in hospitalization and mortality rates between the different TB families.

Discussion

In the past two decades, researchers have focused on developing rapid and sensitive diagnostic approaches to combat TB, as well as molecular epidemiological meth-

Table 2: Families identified in typing and percentages

Family name	n	%	Family name	n	%
T1	161	40	LAM3	1	0.2
T2	15	3.7	LAM3 - S (convergent)	16	4
T1-T2	2	0.5	LAM7 - TUR	28	7
T3	5	1.2	T2-S	2	0.5
T4	2	0.5	LAM9	6	1.5
H1	20	5	Beijing	9	2.2
H2	4	1	CAS	1	0.2
H3	33	8.2	CAS1 - DELHI	3	0.7
H4	10	2.5	X1	2	0.5
U	16	4	T5 - RUS1	1	0.2
S	11	2.7	T1 - RUS2	2	0.5

ods with enhanced discriminatory power. With the development of molecular methods, the typing of MTC strains has become pivotal in answering important epidemiological questions. These questions include identifying the source of infection in communities or families and understanding its spread. This approach also supports the early detection of resistant strains. Molecular follow-up studies enable the determination of prevalence and transmission routes of different species and genotypes in different geographical regions around the world. This knowledge is crucial in developing strategies for controlling TB and measuring the success of existing control programs.

Globally, 86% of patients with active TB reside in developing countries, where 95% of TB-related deaths also occur. Over the past three decades, many developed countries have seen the rise in tuberculosis incidence. This increase is attributed to several factors, including socioeconomic problems, immigration, neglect of TB control programmes, mass migration due to regional wars or internal disturbances, and particularly the emergence of the Human Immunodeficiency Virus / Acquired Immune Deficiency Syndrome (HIV / AIDS) epidemic. Inadequate control programmes have led to the prevalence of drug resistance. Some genotypes, such as the multidrug-resistant Beijing family, have spread beyond their original regions, posing a significant global public health threat.[7]

There have been several studies analyzing different species and genotypes within the MTC using molecular techniques. Lari et al.[8] evaluated 248 MTC isolates collected from hospitalized patients in Tuscany, Italy, over one year using the spoligotyping method. They reported that 116 isolates were single-member unique clusters, 12 isolates were unidentified, and 166 isolates formed 34 clusters (67%). The authors identified the T1 family (11.6%), the H3 family (7.2%), and the LAM9 family (5.2%) as the three most predominant spoligotype families in Tuscany. They also detected seven (2.8%) strains belonging to the Beijing family.

Molina-Torres et al.[9] conducted a study in Monterrey, Mexico, in 2010, during which 180 MTC isolates were genotyped by spoligotyping. The authors reported that the most common gene family was T1 with 43 (23.8%) strains, while the second most common was X1 with 28 (15.5%) strains. Our study identified the T1 family as the most common family in our region, which concurs with these findings.

In 2013, Al-Hajoj et al.[10] conducted a study in Saudi Arabia of 902 clinical isolates using the spoligotyping and MIRU-VNTR methods. They identified Delhi / Central Asian (CAS) (26.4%), East-African Indian (EAI) (13.7%), and Haarlem (11.3%) as the most common groups, respectively. This study reported a lower frequency of the H, LAM, and TUR families, which were more common in our analyses, while

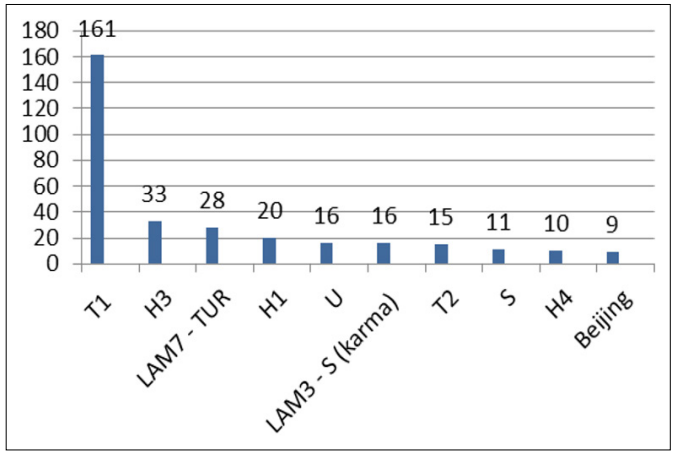

Figure 1: Ranking of the top 10 families

Table 3: Distribution of demographic characteristics, comorbidities, and regions of patients by family groups

	T1		P3		LAM7-TUR		P1		U		LAM3-S (convergent)		T2		S		P4		Beijing		p
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Gender (Female)	69	42.9	12	36.4	10	35.7	8	40	6	37.5	8	50	6	40	3	27.3	3	30	5	55.6	0.93
Age (years) (mean±SD)	54±17.4		48±21.1		47±15.8		55±15.4		42±20.2		59±17.8		51±16		43±17.8		56±19.8		45±24.4		**0.02**
Region																					0.04
Aegean	88	55	13	39.4	13	46.4	13	65	9	56.3	9	56.3	7	46.7	4	36.4	3	30	1	11.1	
Mediterranean	10	6.3	2	6.1	0	0	1	5	0	0	1	6.3	1	6.7	0	0	2	20	0	0	
Marmara	15	9.4	4	2.1	5	17.9	0	0	2	12.5	0	0	3	20	2	18.2	0	0	2	22.2	
Central Anatolia	10	6.3	3	9.1	1	3.6	1	5	2	12.5	1	6.3	0	0	0	0	1	10	2	22.2	
Black Sea	8	5	4	12.1	0	0	2	10	0	0	1	6.3	1	6.7	3	27.3	1	10	0	0	
Eastern Anatolia	15	9.4	6	18.2	6	21.4	2	10	2	12.5	3	18.8	3	20	0	0	3	30	1	11.1	
Southeastern	6	3.8	0	0	1	3.6	1	5	1	6.3	0	0	0	0	2	18.2	0	0	0	0	
Foreign	8	5	1	3	2	7.1	0	0	0	0	1	6.3	0	0	0	0	0	0	3	33.3	
Comorbidities																					1.0
No comorbidity	95	59	24	72.7	20	71.4	11	55	11	68.8	9	56.3	10	66.7	8	72.7	7	70	7	77.8	
Malignancy	20	12.4	2	6	2	7.1	2	10	1	6.3	0	0	3	20	1	9.1	1	10	0	0	
Diabetes	13	8.1	2	6	2	7.1	3	15	1	6.3	3	18.7	2	13.3	0	0	2	20	1	11.1	
CKD	3	1.9	1	3	1	3.6	0	0	0	0	2	12.5	0	0	2	18.2	0	0	0	0	
Smoking	54	36.7	12	36.4	11	40.7	6	30	8	50	2	12.5	6	40	1	9.1	5	50	1	11.1	0.17
Alcohol	7	4.6	1	3	1	3.7	0	0	1	6.3	1	6.3	0	0	0	0	1	10	0	0	0.9
Immunodeficiency	37	23.1	3	9.1	5	18.5	4	20	4	25	2	12.5	3	20	3	27.3	2	20	1	11.1	0.8
Previous TB	8	5.1	2	6.3	2	7.4	2	11.1	0	0	1	6.7	2	14.3	0	0	1	11.1	1	14.3	0.7

CKD: Chronic Kidney Diseases

the T1 family, the most common in our study, was not detected. A statistically significant correlation was observed between patient gender and the EAI and LAM families (EAI; p=0.026 and LAM; p=0.005). Our study found no significant age and gender differences between the families.

In a 2014 study by Jiang et al.,[11] 180 clinical MTC strains from China were genotyped by spoligotyping. Of these strains, 92 were classified as Beijing, 28 as Undefined (U), 13 as T, 11 as MANU, five as Haarlem, four as Central Asian (CAS), and two as the LAM families. While the T1 family, the most common in our study, ranked third, the Beijing family, the tenth most common in our study, was identified as the most common in the Jiang et al. study. This finding is considered an indicator of the geographical differences in genotyping. The study by Mbugi et al.,[12] conducted in Tanzania in 2015, reported that 55 (25.7%) of the *M. tuberculosis* isolates belonged to the CAS family, 52 (24.3%) to the T family, and 38 (17.8%) to the LAM family. Of the LAM family, 25 (11.7%) belonged to the EAI family, 25 (11.7%) to non-typed families, and 8 (3.7%) to the Beijing family. A minority group, including Haarlem, X, U, and S families, accounted for 11 (5.2%) of all genotypes. The CAS family, which was the most common in the said study, was among the least identified families in the present study.

There have been only a limited number of studies conducted in our region monitoring MTC members using molecular epidemiological methods. The results of such studies, as expected, revealed the Beijing genotype to be reported in only limited numbers in our Western neighbors, while the incidence in our Eastern neighbors is increasing.

In 2008, Valcheva et al.[13] evaluated 113 *M. tuberculosis* strains isolated from different regions of Bulgaria to identify their genotypic characteristics using spoligotyping and MIRU-VNTR methods. They detected 15 clusters, with the largest cluster belonging to the T1 family strains (25.7%), followed by the LAM7-TUR family (5.4%). The authors noted that the prevalence of LAM7-TUR, above the global average, could be attributed to its proximity to Türkiye, highlighting the significance of migration in the spread of *M. tuberculosis* genotypes.

Table 4: Distribution of radiological and laboratory findings among patient groups by family classification

	T1		P3		LAM7-TUR		P1		U		LAM3-S (convergent)		T2		S		P4		Beijing		p
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Pulmonary TB	104	64.6	27	81.8	19	67.9	12	60	10	62.5	10	62.5	9	60	6	54.5	5	50	7	77.8	0.6
EP-TB organ involvement																					**0.03**
Lymph nodes	24	38.1	4	36.4	5	50	2	20	2	28.6	2	33.3	1	16.7	1	20	3	60	0	0	
Pleura	14	22.2	4	36.4	2	20	2	20	0	0	1	16.7	0	0	1	20	0	0	1	50	
Bones–Joints	6	9.5	0	0	1	10	0	0	2	28.6	1	16.7	1	16.7	0	0	0	0	0	0	
ARB																					0.3
Negative	99	61.5	22	66.7	19	67.9	11	55	6	37.5	10	62.5	9	60	6	54.5	7	70	3	33.3	
1 (+)	16	9.9	5	15.2	2	7.1	4	20	3	18.8	5	31.3	2	13.3	2	18.2	1	10	1	11.1	
2 (+)	14	8.7	3	9.1	1	3.6	1	5	3	18.8	1	6.3	1	6.7	0	0	1	10	3	33.3	
3 (+)	8	5	1	3	3	10.7	3	15	0	0	0	0	0	0	1	9.1	1	10	1	11.1	
4 (+)	24	14.9	2	6.1	3	10.7	1	5	4	25	0	0	3	20	2	18.2	0	0	1	11.1	
Radiological findings																					
Cavitary lesion	45	28.3	6	18.8	9	33.3	3	15	3	18.8	1	6.3	2	13.3	2	18.2	1	10	1	12.5	0.3
Multilobar involvement	47	29.7	7	21.9	4	14.8	8	40	7	43.8	4	25	1	6.7	5	45.5	1	10	3	37.5	**0.05**
Pleural fluid	28	17.4	7	21.9	3	11.1	3	15	0	0	2	12.5	0	0	1	9.1	1	10	1	12.5	0.4
Leukocytes ($10^3/\mu L$) (mean±SD)	8.02±3.73		8.8±4.81		8.47±3.72		7.87±2.45		7.53±1.99		7.22±2.63		7.58±2.67		5.8±1.74		7.54±3		9.39±3.61		0.3
Hb (g/dL) (mean±SD)	11.7±1.9		11.9±1.7		13±1.6		11.3±2.1		11.8±1.6		11.2±1.7		11.2±1.8		11.6±2.1		12.6±1.8		11±1.8		**0.04**
CRP (mg/dL) (mean±SD)	6.9±7.04		8.3±10.4		5.3±7.3		5.2±5.2		4.2±4.3		4.6±3.9		7.4±5.6		5.6±5.7		10±13.2		4.9±6.7		0.7

EP-TB: Extrapulmonary TB, ARB: Asid Resistance Bacillus, Hb: Hemoglobin

Rohani et al.[14] evaluated 113 *M. tuberculosis* isolates collected from the Iran-Khorasan region in 2009, reporting that the largest cluster consisted of a local pattern of 13 strains, while the Beijing genotype rate was 7.1%. Nieman et al.[15] examined the distribution of the Beijing family in Georgia in 2010 and evaluated 183 *M. tuberculosis* isolates by spoligotyping. They found that 26% of the evaluated strains belonged to the Beijing family, 18% to the LAM family, 12% to the Ural family, and 5% to the Haarlem family.

Although the data are limited, they indicate that the incidence of *M. tuberculosis* Beijing strains is increasing in our region as well as globally.

A study conducted in our country in the 2000s profiled 147 *M. tuberculosis* strains from Malatya and 98 strains from Ankara using spoligotyping. In Malatya, more than 58% of the patients, and in Ankara, more than 38%, were associated with four types: LAM7-TUR and the T1 superfamily, H, and an undesignated family. Regarding the distribution of these types in Türkiye and worldwide, LAM7-TUR was predominant in Türkiye, while T1 and H were equally distributed both in Türkiye and other parts of the world. LAM7-TUR has been identified as a new *M. tuberculosis* clone specific to Türkiye. The H and poorly-defined T superfamily have been reported as the major spoligotype families identified in Türkiye. The Beijing type was also observed in a small number in this study, although the authors did not consider it a significant problem for the country yet.[16]

In our country, a study conducted by Zeytinli et al.[17] in Adana applied the MIRU-VNTR method to evaluate samples taken from pulmonary TB patients who presented to the Adana Regional Tuberculosis Laboratory between January 2007 and June 2010. The findings indicated that the T1 family was the most common in the region, accounting for 239 (51.9%) isolates, followed by the LAM7-TUR family with 54 (11.5%) isolates. Only six (1.3%) of the strains from Şanlıurfa were multidrug-resistant isolates belonging to the Beijing family.

In Africa, a region with countries at high risk of *M. tuberculosis*, Asiimwe et al.[18] an-

Table 5: Distribution and proportions of families by years of isolation

Family	Year of isolation											
	2009		2010		2011		2012		2013		2014	
	n	%	n	%	n	%	n	%	n	%	n	%
T1	39	24.2	35	21.1	21	13	21	13	18	11.2	28	17.4
H3	4	12.1	7	21.2	10	30.3	4	12.1	2	6.1	6	18.2
LAM7-TUR	9	32.1	5	17.9	6	21.4	3	10.7	4	14.3	1	3.6
H1	3	15	3	15	2	10	4	20	2	10	6	30
U	1	6.3	3	18.8	3	18.8	3	18.8	4	25	2	12.5
LAM3-S (convergent)	1	6.3	4	25	4	25	4	25	0	0	3	18.8
T2	4	26.7	0	0	3	20	6	40	1	6.7	1	6.7
S	2	18.2	1	9.1	1	9.1	3	27.3	2	18.2	2	18.2
H4	2	20	2	20	1	10	1	10	1	10	3	30
Beijing	1	11.1	2	22.2	2	22.2	0	0	3	33.3	1	11.1

Table 6: Distribution of drug resistance among families

Family	Drug resistance									
	R		H (0.2 µg/ml)		H (1 µg/ml)		S		E	
	n	%	n	%	n	%	n	%	n	%
T1	1	0.7	7	4.8	2	1.3	7	4.6	3	2.3
H3	1	3.1	5	15.6	2	6.3	5	15.6	2	7.7
LAM7-TUR	1	3.6	9	32.1	4	14.3	2	7.1	0	0
H1	0	0	1	8.3	0	0	1	5	0	0
U	0	0	3	27.3	2	20	0	0	1	8.3
LAM3-S (Convergent)	0	0	2	12.5	2	12.5	1	6.3	1	6.7
T2	0	0	0	0	0	0	0	0	0	0
S	1	9.1	1	9.1	1	9.1	1	9.1	0	0
H4	0	0	1	16.7	0	0	1	10	0	0
Beijing	**5**	**55.6**	**5**	**55.6**	**5**	**55.6**	**5**	**55.6**	1	14.3

p≤0.001

alyzed 344 TB strains isolated by spoligotyping. They reported the most common families to be T2 (70%, 241 strains), CAS1-Kili (3.5%), LAM9 (2.6%), and CAS1-Delhi (2.6%). The Beijing genotype was found in 1.2% (4 strains) of the cases. Additionally, 26.7% of the strains were isolated from HIV seropositive patients, with 8.1% showing resistance to H and 4.4% to R. In the present study, four of the 12 HIV seropositive patients were from the T1 family, with no strains belonging to the Beijing family.

In a 2014 study by Zhang et al.[19] in China, the percentages of streptomycin-resistant, kanamycin-resistant, and Extensively Drug-Resistant (XDR) TB in the modern Beijing genotype were reported to be significantly lower than in the older genotype (p<0.05). Among the 376 multidrug-resistant TB isolates, 261 strains (69.4%) belonged to the Beijing genotype, while the remaining 115 (30.6%) belonged to non-Beijing genotypes. Resistance to streptomycin (S) was detected in 73.6% of the Beijing strains, and ethambutol resistance in 52.1%. The rate of multidrug-resistant strains in the Beijing genotype was found to be significantly higher (p<0.01) than that in the non-Beijing strains.

In 2014, Cáceres et al.[20] conducted a study on a total of 142 XDR-TB *M. tuberculosis* complex strains, where each strain was analyzed using DNA spoligotyping and MIRU-VNTR methods. The most common family identified was Haarlem (43.6%), followed by T1 (27.4%), LAM (16.2%), Beijing (9.1%), and X (1.4%). Regarding the drug-resistant profile, the authors found that 62.6% were resistant to capreomycin (CAP) and kanamycin (KAN), which are injectable agents that can be administered alone (15.5% for CAP, 21.8% for KAN). Our study did not include an examination of these drug groups.

In 2016, Li et al.[21] conducted a study in China, genotyping a total of 298 *Mycobacterium tuberculosis* clinical isolates collected over one year from patients with smear-positive pulmonary TB. This genotyping used spoligotyping and 15-locus VNTR methods. The authors identified the Beijing family lineage as the most prominent (81.54%, 243/298), followed by other family lineages such as the T family (9.06%, 27/298), U family (0.67%, 2/298), LAM9 family (0.34%, 1/298), and Manu family (0.34%, 1/298). No statistically significant differences were found in multidrug-resistant (MDR) *M. tuberculosis*, age, case type, or education rates between the Beijing and non-Beijing family strains.

In our general observation of global studies, the types and distributions of families were found at the expected rates for our country's geography (with T1 and LAM7-TUR being among the most common families). Regarding drug resistance, the Beijing strain was identified as the most common, aligning with findings in existing literature. Although it is an uncommon strain for our region, it was found at negligible rates, which can be attributed to migrations and genetic transitions. Our study examined only the four TB drugs for drug resistance and did not analyze other drug groups. Additionally, our aim was to evaluate patients regarding their BCG status, Quantiferon tests, and results, but we were unable to access sufficient patient information. These can be considered limitations of our study.

Unlike previous studies in the literature, our study also compared patients' smoking habits, alcohol use, comorbidities, clinical findings, radiological and laboratory findings, hospitalization, and mortality rates. Furthermore, we evaluated not only pulmonary TB cases but also EP-TB cases. These aspects can be considered strengths of our study, although no significant difference was found in these parameters. We intended to evaluate the patients concerning their BCG status, Quantiferon tests, and results, but could not access sufficient patient information. Additionally, the low number of patients coming from abroad through immigration did not permit a statistical assessment.

Conclusion

Our evaluations reveal that, unlike earlier reports from our country, strains belonging to the T1 family were more common, rather than the LAM7-TUR and the Beijing groups. The latter, which has been identified with multidrug resistance, was observed more than expected (2.2%). This increase was believed to result from both migration and genetic transmission. A comparison of families' smoking habits, alcohol use, comorbidities, clinical findings, radiological and laboratory results, hospitalization, and mortality rates revealed no significant differences.

It is believed that spoligotyping reveals the movement of the MTC within the region, particularly in large regional epidemics, and thus can be extremely useful in developing prevention and control strategies, as well as in determining the success of applied strategies. Its use in combination with MIRU-VNTR would enable more detailed, albeit small-scale, projections from an epidemiological standpoint. Molecular epidemiological studies, with completed infrastructures, can be repeated routinely or in larger patient samples, contributing significantly to monitoring movements in our region and controlling MTC infections. Further studies are needed to evaluate the potential genotypic differentiation in TB patients that occurs due to the social and ethnic changes brought about by the recent migration wave in our country.

Ethics Committee Approval

The study was approved by the Ege University Clinical Research Ethics Committee (No: 16-12.1/5, Date: 06/01/2017).

Authorship Contributions

Concept – İ.T., C.Ç., M.H.Ö.; Design – İ.T., C.Ç., M.H.Ö.; Supervision – İ.T., C.Ç., M.H.Ö.; Funding – İ.T., C.Ç., M.H.Ö.; Materials – İ.T., C.Ç., M.H.Ö.; Data collection &/or processing – İ.T., C.Ç., M.H.Ö.; Analysis and/or interpretation – İ.T., C.Ç., M.H.Ö.; Literature search – İ.T., C.Ç., M.H.Ö.; Writing – İ.T., C.Ç., M.H.Ö.; Critical review – İ.T., C.Ç., M.H.Ö.

Conflicts of Interest

There are no conflicts of interest.

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