



# Investigation of the Sensitivity of *Mycobacterium tuberculosis* Strains Isolated from Various Clinical Samples in Eastern Turkey to Major Anti-tuberculosis Drugs

## Türkiyenin Doğusunda Çeşitli Klinik Örneklerden İzole Edilen *Mycobacterium tuberculosis* Suşlarının Major Anti-Tüberküloz İlaçlara Duyarlılıklarının Araştırılması

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### Abstract

**Objective:** In the study, 419 *Mycobacterium tuberculosis* complex strains, which were isolated from samples sent to the Regional Tuberculosis Laboratory in our city between 2015 and 2019 with suspected tuberculosis from Erzurum and surrounding cities were investigated.

**Material and Method:** Our research is a retrospective cross-sectional study and clinical samples sent to the Regional Public Health Laboratory with suspicion of Tuberculosis within a five-year period from January 2015 to December 2019 were first evaluated by using Ehrlich-Ziehl-Neelsen staining technique. The samples were then simultaneously inoculated into the automated culture system (BACTEC MGIT 960) with Löwenstein-Jensen medium. Sensitivity research was conducted for isoniazide (INH), rifampicin (RIF), streptomycin (STM) and ethambutol (ETM) antibiotics with BACTEC MGIT 960 system to samples defined by *M. tuberculosis* complex strains identification test (TbC ID) from reproduction positive tubes.

**Results:** In our study, 337 (80.4%) of the 419 *M. tuberculosis* complex strains investigated in our study were susceptible to all major anti-TB drugs, while 82 (19.6%) were found to be resistant to at least one major anti-TB drug. The distribution of resistance ratios was INH (11.9%), STM (11.7%), RIF (4.1%), ETM (3.6%). Multi-drug resistant (MDR)-TB ratio (IZN+ RIF) was found to be (3.6%).

**Conclusions:** In our study, in the strains in which resistance to anti-TB drugs was investigated, the highest drug resistance was determined for IZN. The lowest drug resistance was determined for ETM.

**Keywords:** *Mycobacterium tuberculosis*, drug resistance, multiple drug resistant (MDR) tuberculosis, anti-tuberculosis drugs

### Öz

**Amaç:** Çalışmada ilimizde bulunan Bölge Tüberküloz Laboratuvarı'na 2015-2019 yılları arasında Erzurum ve çevre illerden tüberküloz şüphesi ile gönderilen örneklerden izole edilmiş olan 419 *Mycobacterium tuberculosis* kompleks suşun, major anti-tüberküloz ilaçlara karşı direnç durumu araştırıldı.

**Gereç ve Yöntem:** Araştırmamız retrospektif kesitsel bir çalışma olup, Ocak 2015-Aralık 2019 tarihleri arası beş yıllık sürede Bölge Halk Sağlığı Laboratuvarına Tüberküloz şüphesiyle gönderilen klinik örnekler, ilk önce Ehrlich-Ziehl-Neelsen boyama yöntemi kullanılarak değerlendirildi. Daha sonra örnekler eş zamanlı olarak Löwenstein-Jensen besiyeri ile otomatize kültür sistemine (BACTEC MGIT 960) ekimleri yapıldı. Üremesi pozitif tüplerden identifikasyon testi (TbC ID) ile *M. tuberculosis* kompleks suşu tanımlaması yapılan örnekler BACTEC MGIT 960 sistemi ile izoniazid (INH), rifampisin (RIF), streptomisin (STM) ve etambutol (ETM) antibiyotikleri için duyarlılık araştırması yapıldı.

**Bulgular:** Çalışmamızda ilaç duyarlılığı araştırılan 419 *M. tuberculosis* kompleks suşun 337'si (%80,4) majör anti-TB ilaçların tümüne duyarlı iken 82'si (%19,6) en az bir majör anti-TB ilaca dirençli bulundu. Direnç oranlarının dağılımı IZN (%11,9), STM (%11,7), RIF (%4,1), ETM (%3,6) idi. Çoklu ilaca dirençli (ÇİD)-TB oranı (INH +RIF) (%3,6) olarak bulundu.

**Sonuç:** Çalışmamızda majör anti-TB ilaçlara karşı direnç araştırılan suşlarda en yüksek ilaç direnci IZN için belirlendi. En düşük ilaç direnci ETM için saptandı.

**Anahtar Kelimeler:** *Mycobacterium tuberculosis*, ilaç direnci, çoklu ilaç dirençli (ÇİD) tüberküloz, anti-tüberküloz ilaçlar



## INTRODUCTION

Tuberculosis (TB) caused by *M. tuberculosis* is a disease that causes the deaths of more than 1 million people each year in mostly low-income and middle-income countries.<sup>[1,2]</sup> TB remains one of the top 10 causes of deaths worldwide. Millions of people continue to fall ill from TB every year.<sup>[3]</sup> There has been a significant decrease in the registered incidence of tuberculosis in Turkey in recent years. As such, while in 2005 the incidence ratio was 29.4 per hundred thousand in 20,535 registered patients, it decreased to 14.6 per hundred thousand in 2017.<sup>[4]</sup> In our country, out of the 12,046 total number of patients diagnosed with TB in 2017, 92.2% were new cases; 7.8% were previously treated cases; 42.3% were female; 57.7% were male; 66.1% were patients with pulmonary involvement; 33.9% were patients with extrapulmonary involvement.<sup>[4]</sup>

The treatment of tuberculosis with drugs began towards the end of the 1940s and over time resistance developed against the drugs used in this treatment. Resistance to anti-TB drugs is an important problem.<sup>[5]</sup> The drug-resistant TB (DR-TB) problem which has attracted attention especially since the early 1990s, has reached a global dimension today. The increase, especially in multi-drug resistant (MDR) TB cases, is the most important problem that threatens the success of TB control programs.<sup>[6]</sup> While drug resistance can develop spontaneously in a drug-sensitive strain before treatment, MDR-TB could also develop despite the fact that the primary treatment is completed.<sup>[7,8]</sup> MDR-TB is defined as TB resistant to caused by *M. tuberculosis* complex isolates resistant to INH and RIF which are among the primary (first-line) anti-TB drugs.<sup>[9,10]</sup> In our country, the number of MDR-TB in 2017 patients was 191. In 2015, the treatment success for MDR-TB patients at the 24<sup>th</sup> month 2015 was 67.8%.<sup>[4]</sup> The American Thoracic Society and the Center for Disease Control and Prevention (CDC) recommend susceptibility tests for at least first step anti-TB drugs in all older and new TB cases to ensure that patients with TB can be treated correctly and prevent the development of resistance to anti-TB drugs.<sup>[6]</sup>

In this study, resistance to INH, RIF, STM and ETM of 419 *M. tuberculosis* isolates isolated from different clinical specimens with suspected tuberculosis from 11 provinces in Erzurum and its surrounding were investigated.

## MATERIAL AND METHOD

In our research, sensitivity results for STM, INH, ETM, RIF against major drugs used in TB treatment in 419 *Mycobacterium tuberculosis* complex strains isolated from patient clinical samples sent to the Regional Public Health Laboratory with pre-TB diagnosis between 2015 and 2019 from 13 cities, mainly from cities in eastern Anatolia region were investigated. All operations in the laboratory were performed in Class II biosafety cabins using the necessary protective personal equipment such as gloves, glasses and protective clothing. If different clinical samples had been

sent for a patient, only one strain isolated from among these samples was included in the study. Multiple antibiotic sensitivity tests were performed for a patient in one year and if the MDR- *M. tuberculosis* complex strain was not defined in subsequent clinical samples, the first result was included in the study. Whether the patients who participated in the study had previously used anti-TB drugs, could not be definitively revealed because of the changes in our hospital automation systems at different times and the shortcomings in patient records.

Pleural fluid and Cerebrospinal fluid (CSF) samples coming to the laboratory which were thought to have been obtained in aseptic conditions, were processed directly without being decontaminated; while clinical samples such as sputum, abscess, bronchial lavage were processed after homogenization and decontamination using N-acetyl-L-cysteine and sodium hydroxide (NALC and NaOH). Then the pH of the clinical sample was adjusted, and it was centrifuged and intensified. The resulting sediment was simultaneously inoculated in 0.5 ml MGIT tubes (Becton Dickinson Diagnostics, USA), which contain Middlebrook 7H9 liquid medium used simultaneously in both the Löwenstein-Jensen classical culture medium and BACTEC MGIT 960 full automatic systems. 0.8 ml PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin) was added to MGIT tubes before inoculation.

All samples where inoculation was done were left for incubation for 42 days. Acid resistant staining was made in samples that gave positive results. BD MGIT-TBC Identification Test (TBc ID) (Becton Dickinson Diagnostics, USA) detecting MPT64 antigen was administered in accordance with the recommendations of the manufacturer. Sensitivity/resistance to major anti-TB drugs of the strains evaluated as *M. tuberculosis* complex strain was investigated in accordance with the manufacturer's operating procedure using BACTEC MGIT 960 system to be the final concentration of the drug being ETM (5.0 µg/ml), INH (0.1 µg/ml), RIF (1.0 µg/ml), and STM (1.0 µg/ml).

In addition to using H37Rv reference strain (ATCC 35838) during the laboratory internal quality control process where the research was carried out, our laboratory is also included in the external quality program of the Turkish Public Health Laboratory of the Ministry of Health. This research was approved by the "Atatürk University Faculty of Medicine Ethics Board of Clinical Research of " by decree no. 19 on 13.02.2019.

## Statistical Analysis

In the analysis of the data used in the study the 22.0 IBM SPSS package program was used. The frequency and percentages of the data was calculated. When evaluating the results of categorical data in our research, A p- value equal or less than 0.05 ( $\leq 0.05$ ) is considered statistically significant.

## RESULT

A total of 419 strains isolated from Erzurum, Ağrı, Erzincan, Kars, Batman, Mardin, Siirt, Rize, Ardahan, Giresun, Bayburt and Iğdır cities were included in the study between January 2015 and December 2019. The strains were isolated from various clinical materials in the tuberculosis unit of Erzurum Regional Public Health Laboratory. The distribution of clinical samples included in this study are also shown in **Table 1**. It was observed that 259 (61.8%) of patients from whom *M. tuberculosis* complex strain was isolated were male and 160 (38.2%) were female. The distribution of strains taken by year consisted of 61 strains in 2015, 62 in 2016, 106 in 2017, 113 in 2018 and 77 in 2019.

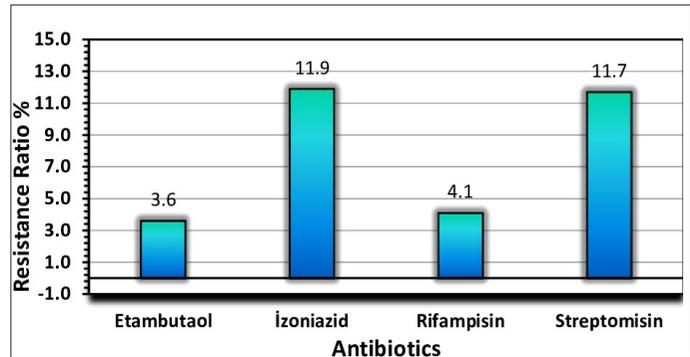
Resistance ratios of strains isolated using BACTEC MGIT 960 method against anti-tuberculosis (anti-TB) drugs are shown in **Table 2** according to gender. 337 (80.4%) of the strains were susceptible to all major anti-TB drugs, while 82 (19.6%) were found to be resistant to at least one major anti-TB drug. The highest resistance ratio was found to be 11.9% against INH, while the lowest resistance ratio was determined to be 3.6% against ETM antibiotics (**Figure 1**). Resistance to STM was 11.7%, resistance to RIF was 4.1% (Figure 1). In our study, while there was single resistance in 51 (12.2%) of the isolates, in 31 (7.4%) of the isolates there was resistance to two or more of the tested drugs (**Table 3**). MDR-TB ratio (IZN+RIF) has been determined 15 (3.6%) isolates. In this study 6 (1.4%) isolates resistant to all four of the antibiotics (IZN+RIF+ETM+STM) were identified. *M. tuberculosis* isolate were found in 200 (47.7%) of all 419 specimens submitted for microscopy.

**Table 1.** Samples from which *Mycobacterium tuberculosis* complex strain was isolated

Patient sample	Number (n)	Percentage (%)
Sputum	352	84
Broncho alveolar lavage	37	8.8
Aspiration material	15	3.6
Pleural Fluid	11	2.6
Biopsy	3	0.7
Peritoneal fluid	1	0.2
Total	419	100

**Table 2.** State of resistance to antibiotics depending on gender status

Characteristic	Antibiotic Resistance Positive		P value
	Number (n)	Percentage (%)	
Gender			
Male (n=259)	56	21.6	0.206
Female (n=160)	26	16.3	
Total	82	19.6	



**Figure 1.** Resistance ratios against major anti-tuberculosis drugs

**Table 3.** The state of resistance of examined *Mycobacterium tuberculosis* complex strains against major anti-tuberculosis drugs according to years

Characteristic	2015	2016	2017	2018	2019	Toplam
	n (%)					
Tested isolate	61 (100)	62 (100)	106 (100)	113 (100)	77 (100)	419 (100)
Sensitive to anti-TB drugs	50 (82.0)	56 (90.3)	86 (81.1)	83 (73.5)	62 (80.5)	337 (80.4)
Resistance to any drug	11 (18.0)	6 (9.7)	20 (18.9)	30 (26.5)	15 (19.5)	82 (19.6)
STM	4 (6.6)	5 (8.1)	10 (9.4)	23 (20.4)	7 (9.1)	49 (11.7)
INH	11 (18.0)	3 (4.8)	13 (12.3)	14 (12.4)	9 (11.7)	50 (11.9)
RIF	5 (8.2)	1 (1.6)	4 (3.8)	4 (3.5)	3 (3.9)	17 (4.1)
EMB	1 (1.6)	1 (1.6)	5 (4.7)	5 (4.4)	3 (3.9)	15 (3.6)
Resistance to single drug	4 (6.6)	4 (6.5)	12 (11.3)	19 (16.8)	12 (15.6)	51 (12.2)
STM	0 (0)	3 (4.8)	3 (2.8)	15 (13.3)	4 (5.2)	25 (6.0)
INH	4 (6.6)	1 (1.6)	6 (5.7)	4 (3.5)	6 (7.8)	21 (5.0)
RIF	0 (0)	0 (0)	1 (0.9)	0 (0)	0 (0)	1 (0.2)
EMB	0 (0)	0 (0)	2 (1.9)	0 (0)	2 (2.6)	4 (1.0)
Resistant to multiple drugs (MDR)	5 (8.2)	1 (1.6)	3 (2.8)	3 (2.7)	3 (3.9)	15 (3.6)
INH+RIF	3	0 (0)	0 (0)	0 (0)	1 (1.3)	4 (1.0)
INH+RIF+EMB	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
INH+RIF+SM	1 (1.6)	0 (0)	1 (0.9)	2 (1.8)	1 (1.3)	5 (1.2)
INH+RIF+EMB+SM	1 (1.6)	1 (1.6)	2 (1.9)	1 (0.9)	1 (1.3)	6 (1.4)
Other resistance patterns	2 (3.3)	1 (1.6)	4 (3.8)	8 (7.1)	1 (1.3)	16 (3.8)

INH: Isoniazide, RIF: Rifampicin, EMB: Ethambutol, STM: Streptomycin

## DISCUSSION

Epidemiological data and lab data are important in terms of evaluating achievements in the fight against tuberculosis, obtaining healthy data and determining real resistance ratios. The data obtained in our study is important for regional evaluation as it includes Erzurum city and surrounding cities. When we look at the resistance ratios of at least one anti-TB drug in some studies conducted in Turkey and around the world; Esenkaya Taşbent et al.<sup>[11]</sup> found resistance to at least one major drug in 16.9% of patients. In the study by Burak Selek et al.<sup>[12]</sup> 27.8% of the isolates obtained from patients were found to be resistant to at least one antibiotic. In their study, Artan et al.<sup>[9]</sup> reported that, 25.5% of the strains isolated from older and new TB patients were resistant to at least one of the anti-TB drugs. In Ethiopia, Adene et al.<sup>[13]</sup> found 20.2% resistance to any primary anti-TB drug. In Korea, 19.4% of isolates taken from patients in Lee et al.<sup>[14]</sup> study group. Were shown to be resistant to any one of the anti-TB drugs. In He et al.<sup>[15]</sup> study in China, the resistance rate against at least the first step drug was 21.2%. According to the results of our study, the resistance rate against any major anti-TB drug was 19.6%.

In the "Tuberculosis War in Turkey 2018 Report" published by the Ministry of Health in Turkey, resistance ratios against major anti-TB drugs in 2009-2016 were reported to be 11.9-15.4% for INH; 4.2-6.8% for RIF; 3.7% to 5.4% for EMB; 8.5%-11.3% for STM. The first option with the highest resistance ratio in this data is the anti-TB drug INH.<sup>[16]</sup> In a study conducted by Maurya et al. in India, anti-TB resistance ratios were reported as 27.6% for INH, 14.6% for RIF, 14.6% for ETM, 13.8 for SM; In China, Chang et al.<sup>[18]</sup> reported resistance ratios of 11.7% for INH, 2.8% for RIF, 2.5% for ETM, 11.1% for STM; in Korea Lee et al.<sup>[14]</sup> found total resistance ratios of 15.5% for INH, 9.3% for RIF, 6.7% for ETM, 5.4% for STM in old and new cases. Previously in Erzurum, Aktaş et al.<sup>[19]</sup> found resistance ratios of 16.7% for INH, 10% for RIF, 11.7% for STM, 8.3% for EMB against primary anti-TB drugs in their study which investigated the profile of resistance to drugs in two different ways using the by MGIT method. In our study, the average resistance ratios for five years in isolated strains were determined as 11.9% for INH, 4.1% for RIF, 11.7% for STM and 3.6% for EMB. MDR-TB rate was found to be 3.6%. In our study, the highest resistance among major anti-TB drugs was determined for INH, the second highest resistance was found for SM, and the lowest resistance rate was found for EMB antibiotics. The results we obtain are compatible with the Turkish data. According to the previous study of Aktaş et al.<sup>[19]</sup> there has been a decrease in resistance ratios against other antibiotics, excluding resistance to STM.

In many studies conducted around the world outside Turkey, it also was observed that the most common resistance in *M. tuberculosis* complex strain was against INH. Such high resistance to INH and STM is due to the widespread use of this drug in prophylaxis and treatment. It is also assessed that the widespread use of streptomycin for non-TB reasons may be one of the reasons for the high resistance ratio against this

drug.<sup>[15]</sup> We see some differences in the results of the studies carried out in Turkey and in different parts of the world. We think these differences stem from the characteristics of the patient population, their adaptation to treatment and regional differences. However, although there is no homogeneous distribution of resistance ratios between countries or in different parts of the same country, we can say that we are facing a serious resistance problem for tuberculosis.

In the "Tuberculosis War in Turkey 2018 Report", the MDR TB rate was reported as 3.3-5.4% in 2009-2016.<sup>[16]</sup> In the studies on MDR tuberculosis worldwide, quite different MDR-*Mycobacterium tuberculosis* complex strain resistance ratios have been reported. In Japan Hattori et al.<sup>[20]</sup> reported a very low ratio of 0.2% in their study; in Indonesia, Lisdawati et al.<sup>[21]</sup> reported MDR- *M. tuberculosis* complex strain rate as 5.4%, in their study; in Ethiopia, Solomon et al.<sup>[22]</sup> reported a MDR rate of 2.27% in society, while the MDR rate for inmates living in prison was 15.89%; in China, He et al.<sup>[15]</sup> reported a MDR- *M. tuberculosis* complex strain ratio of 6.2%. In a well-attended study; while in Pakistan Akhtar et al.<sup>[23]</sup> reported a quite high resistance ratio of 69% in their study.

In our study, the resistance against MDR- *M. tuberculosis* complex strain was 3.6%, and our results were within the Turkish average. However, an increase in MDR drug resistance rates was observed in Erzurum compared to the results of the previous study by Ozmen et al.<sup>[24]</sup> MDR-TB cases in TB treatment prolong the duration of treatment and reduce the success of treatment. Therefore, the detection of MDR-TB cases in a short time and treating them with appropriate drugs will contribute to the control of TB disease by breaking the chain of transmission. The development of resistance to RIF alone in patients is rarely seen.<sup>[6]</sup> In our study, RIF resistance alone was detected in only one patient (0.2%) over a five-year period. In addition, resistance to all major anti-TB drugs was observed in 6 strains.

There were some limitations to this study. The laboratory we first studied started to serve in 2014 and the study was done on clinical samples sent from hospitals in different cities. For this reason, limited data about patients was reached. Since the patient's past data could not be reached, no assessment could be made about whether the cases were new or old and the where the location of the disease involvement was.

## CONCLUSION

In our study the data presented do not seem to suggest that is any increase or decrease in drug resistance over time. In our study, resistance to major anti-TB drugs was determined for INH, which was the highest drug resistance in the strains investigated. The lowest drug resistance was detected for EM. The results are compatible with previous studies in Turkey. We believe that it would be useful to take into account these resistance ratios against anti-TB drugs when planning the treatment of Tuberculosis in patients in our region.

## ETHICAL DECLARATIONS

**Ethic Committee Approval:** This research was approved by the "Atatürk University Faculty of Medicine Ethics Board of Clinical Research of " by decree no. 19 on 13.02.2019.

**Informed Consent:** Informed Consent not needed

**Referee Evaluation Process:** Externally peer-reviewed

**Conflict of interest statement:** The authors declare that they have no conflict of interest.

**Financial Disclosure:** This research received no external funding

**Authors' contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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