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Research article

Evaluation of the resistance patterns of Mycobacterium tuberculosis complex strains to antituberculous drugs

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ABSTRACT

Aim: To determine the resistance profile of Mycobacterium tuberculosis complex (MTBC) strains to first-line antituberculous drugs.

Methods: A total of 138 patients with MTBC growth from 2008-2018 were evaluated retrospectively. The Ehrlich-Ziehl-Neelsen (EZN) staining method was used for direct smear preparations, the BACTEC MGIT 460 TB system the Lowenstein-Jensen medium for culture planting and the BACTEC NAP test for the diagnosis of MTBC. Susceptibility tests were performed using the BACTEC MGIT 460 TB system with the streptomycin, isoniazid, rifampicin and ethambutol (SIRE) kit in accordance with the manufacturer's recommendations.

Results: Of the total 138 tuberculosis (TB) cases, 44 (31.9%) were female and 94 (68.1%) were male. MTBC was most frequently isolated from pulmonary specimens (90.6%). Acid-resistant bacilli (ARB) positivity was detected in 88 (63.8%) samples by EZN staining for culture-positive samples. In our study, without considering single or multiple drug resistance (MDR), total resistance rates in MTBC strains were determined for, isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and streptomycin (SM); 10.1%, 4.3%, 2.9%, and 12.3% respectively. While the susceptibility to all drugs was 82.6%, multiple drug–resistant tuberculosis (MDR-TB) was 2.9%.

Conclusion: These results are important since they are the first data reported from our province regarding the determination of the resistance profile to anti-TB drugs. Resistance rates in our study were very close to the 2016 data average of the Ministry of Health of Turkey. Determination of TB resistance profiles, as well as proper and regular treatment, will contribute to the control of MDR-TB.

Keywords: Mycobacterium tuberculosis, tuberculosis, drug resistance, antituberculous drugs.

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Introduction

Tuberculosis (TB) is a chronic, granulomatous disease caused by *Mycobacterium tuberculosis* complex (MTBC) [1,2]. Although TB mainly affects developing countries, it continues to be a public-health problem all over the world [3]. It is estimated that one-third of the world's population is latently infected with MTBC. The lifetime risk of developing active TB in individuals with latent TB infection is 5-15% [1,4].

Mycobacteria cultures and the microscopic investigation of the presence of acid-resistant bacteria (ARB) with an Ehrlich-Ziehl-Neelsen (EZN) stain are the main diagnostic methods [5].

Some first-line antituberculous (anti-TB) drugs used in the treatment of TB are isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and streptomycin (SM). The Center for Disease Control and Prevention (CDC) and the World Health Organization (WHO) both recommend the combined use of first-line drugs in the initial treatment of TB. However, the problem of bacterial resistance to these anti-TB drugs is an important problem in Turkey, as well as in the rest of the world. Co-resistance of TB strains to INH and RIF, which are first-line anti-TB drugs, is defined as multidrug resistance [5].

The aim of this study was to investigate INH, RIF, EMB and SM resistance rates of MTBC strains isolated from clinical specimens in the Microbiology Laboratories of Bolu Abant İzzet Baysal University Hospital between 2008 and 2018.

Methods

Permission was obtained from the local clinical research and ethics committee (Decision no. 2018/211) for this study. A total of 138 cases of MTBC growth from the samples sent to the medical microbiology laboratory of our hospital for mycobacteria culture from 2008-2018 were retrospectively analyzed. The samples sent to the laboratory were subjected to homogenization and concentration after decontamination with the N-Acetyl-L-Cysteine (NALC)-Sodium Hydroxide (NaOH) method. The EZN staining method was used for ARB detection in direct smear preparations. In accordance with the recommendations of the manufacturer, mycobacteria cultures were inoculated on BACTEC MGIT 460 TB (Becton Dickinson, USA) and Lowenstein-Jensen media. The BACTEC NAP test was used to identify MTBC bacteria with growth in mycobacteria cultures. Susceptibility tests were performed with the BACTEC MGIT 460 TB system (Becton Dickinson, USA) using the streptomycin, isoniazid, rifampicin and ethambutol (SIRE) kit, according to the manufacturer's recommendations. The data are summarized in frequencies and percentages.

Results

Of the total 138 TB cases included in the study, 44 (31.9%) were female and 94 (68.1%) were male, and the mean age was 48.4 ± 19.09 (Figure 1). ARB positivity was detected in 88 (63.8%) of the samples with MTBC culture growth (Figure 2). MTBC culture growths were most frequently isolated from the lungs of patients (90.6%). The Distribution of MTC strains by sample type is shown in Table 1. The monodrug resistance rates were found as 4.3% for isoniazid, 1.4% for rifampicin, 0.7% for ethambutol, and 8% for streptomycin. In our study, without considering single or multiple drug resistance (MDR), total resistance rates in MTBC strains were determined for isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and streptomycin (SM); 10.1%, 4.3%, 2.9%, and 12.3% respectively. While the

susceptibility to all drugs was 82.6%, multiple drug-resistant tuberculosis (MDR-TB) was found 2.9% (Table 2). Of the total 138 TB cases, 128 (92.8%) were new cases while 10 (7.2%) were previously treated cases. One of the previously treated patients was MDR-TB.

Discussion

TB is one of the top ten causes of deaths in the world. In 2017, 1.6 million people were reported to

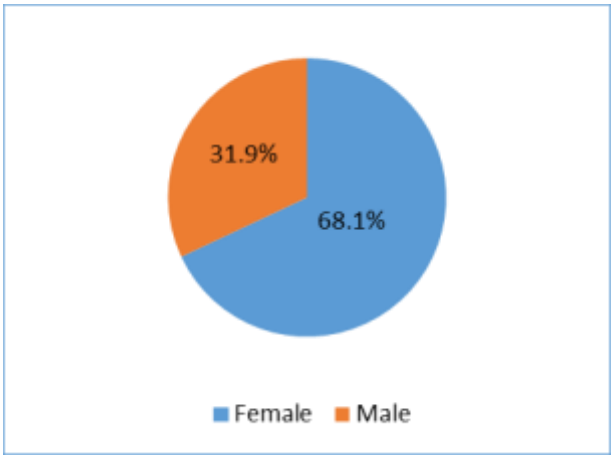

Figure 1. Distribution of TB patients by gender.

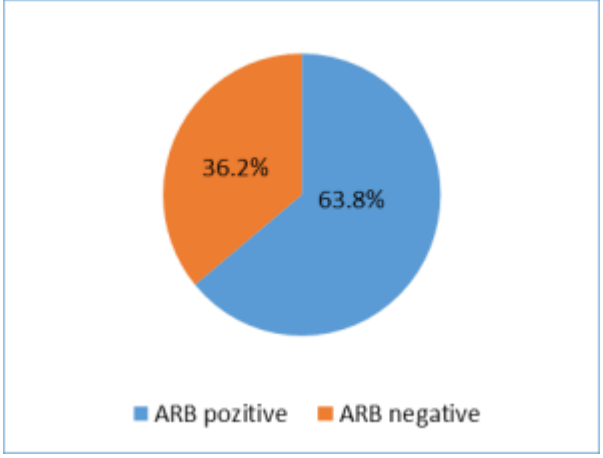

Figure 2. ARB detection rates of samples with MTC growth.

Table 1. Distribution of MTC strains by sample type.

Parameter	Patient sample	n	%
Pulmonary (n=125, %90,6)	Sputum	118	85.5
	Bronchoalveolar lavage	5	3.6
	Fasting stomach juice	2	1.4
Extrapulmonary (n=13, %9,4)	Pleural fluid	4	2.9
	Lymph node	3	2.2
	Biopsy material	2	1.4
	Other (pericardium, bone-joint, meningitis, miliary)	4	2.9
Total		138	100

Table 2. Anti-TB drug resistance rates of MTC strains.

Parameter	Anti-TB drugs	Number of resistant strains n	(%)
*Total resistance for each anti-TB drug	Isoniazide	14	(10.1)
	Rifampin	6	(4.3)
	Ethambutol	4	(2.9)
	Streptomycin	17	(12.3)
Resistance to two drugs	Isoniazide + Rifampin (MDR)	1	(0.7)
	Isoniazide + Streptomycin	3	(2.2)
	Isoniazide + Ethambutol	1	(0.7)
Resistance to three drugs	Isoniazide + Rifampin+ Streptomycin (MDR)	1	(0.7)
Resistance to four drugs	Isoniazide + Rifampin + Ethambutol + Streptomycin (MDR)	2	(1.4)
MDR total		4	(2.9)

*While determining the total number of resistant patients for each drug, it was not considered to be resistant or sensitive to other drugs.

have died from TB [6]. The regions with the highest incidence of TB and the highest TB mortality in the world are Africa and Southeast Asia. Turkey, located in the WHO European region has an estimated incidence of 18 per 100,000 and a mortality rate of 0.62 per 100,000, according to the 2017 Global TB report lower rates than in Europe, which has an incidence rate of 32 per 100,000 and a mortality rate of 3.4 per 100,000 [7].

Clinically, TB is seen in pulmonary (PTB) and extra-pulmonary (EPTB) forms. EPTB is the TB involving organs other than the lungs (e.g., pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, or meninges) [8]. In Turkey, according to the Tuberculosis Control 2018 report, 61.3% of all TB cases were PTB, 33.6% were EPTB, and 5.1% were PTB+EPTB [7]. The results of our study (PTB 90.6%, EPTB 9.4%) differ from this data and instead resemble the results of the study conducted by Çiftçi et al. [9] (PTB 87.4%, EPTB 12.6%) which stated that the EPTB ratio in the European Union and European Economic Area ranged between 4% and 48% in 2011. Difficulties in sampling and diagnosing TB could explain this difference [10].

The determination of acid-resistant bacilli by microscopy is one of the most important methods in the diagnosis of TB and the detection of infectious cases [5]. It is a simple, rapid, inexpensive, and specific method for the diagnosis of TB, but it has a low sensitivity. Direct-preparation sensitivity has been reported to range from 22-78% [11]. These values are known to vary with individual factors, such as, the bacterial genus, the number of bacilli in the sample, decontamination, concentration processes, the spreading thickness, and the experience of the microbiologist [12]. The ARB positivity rate found in our study (63.8%) is similar to the average of the data in our country (59.8%) [7].

Culture is still accepted as the gold standard of TB diagnosis. It only requires 10 tuberculosis bacilli per milliliter of sputum to obtain a positive result from a culture, making it more sensitive than microscopy. It also allows antibiotic susceptibility tests and the identification of mycobacteria species [13].

The most intractable difficulty of TB treatment is the natural drug resistance of MTBC, which has a tough, multi-layered cell wall and effective multi-drug efflux pumps. In addition, it can acquire drug resistance due to inappropriate treatment regimens and various mutations in chromosomal genes caused by treatment noncompliance limiting the availability of an already-small number of drugs [14]. In Turkey's 2018 tuberculosis control report while 78.6% of the strains were susceptible to all drugs tested, the total resistance rates for INH, RIF, SM, and ETM were found 11.9%, 4.2%, 3.7% and 10.6%, respectively [7]. Other Turkish studies found rates ranging between 6.4-20.1% for INH, 4.1-9% for RIF, 72-83.1% for EMB, 2.1-11.1% for SM, and 72-83.1% for all drug-sensitive isolates that have been reported [5,11,15-17]. In our study, while 82.6% of the strains were susceptible to all drugs tested, the total resistance rates for INH, RIF, ETM, and SM were 10.1%, 4.3%, 2.9% and 12.3% respectively which are consistent with the 2016 data average of the Ministry of Health [7]. Consistent with our study in the European region, the sensitivity rate to all first-line drugs was reported to be 85.6% [18]. Second-line anti-TB drugs with more toxic side effects in addition to treatment with first-line drugs, negatively affects both patient compliance and the prolongation of treatment duration [19]. The reasons for the emergence of clinically resistant

TB include the following: treatment errors, the non-use of standard drug regimens, inadequate drug combinations and dosages, irregular drug use, drug additions to a non-healing regimen, low-dose drug use, gastrointestinal absorption problems, and expired or appropriate-condition errors, such as the use of non-stored drugs [20]. According to WHO data, approximately 10.4 million people were infected with TB in 2016. 3.5% of newly diagnosed cases and 18% of previously treated patients are MDR-TB. [16]. Some studies from Turkey found the rate of MDR-TB to be 2.8-5.7% [3,5,11,14-17,21]. The resistance rate of 2.9% found in our study seems to be consistent with the data of the Turkish Ministry of Health 2016 (3.3%) [7].

Conclusion

We think that our results are important since they are the first data reported from our province regarding the determination of the resistance profile against anti-TB drugs. Our resistance rates are very close to the 2016 data average of the Ministry of Health of Turkey. In addition to the diagnosis of TB, the study of susceptibility tests to anti-TB drugs is important for the treatment efficacy. It is known that irregular treatment in patients who have been treated previously can cause MDR-TB development. Therefore, determination of resistance profiles in TB cases, as well as appropriate and regular treatment, will contribute to the control of TB.

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