

RESISTANCE RATES OF MYCOBACTERIUM TUBERCULOSIS COMPLEX ISOLATES OBTAINED FROM CLINICAL SAMPLES TO MAJOR ANTITUBERCULOUS DRUGS: A STUDY IN EDIRNE, TURKEY

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ABSTRACT

Aims: The aim of this study is to reveal the rates of the Mycobacterium tuberculosis complex resistance to major antituberculous drugs (streptomycin, isoniazid, rifampicin, ethambutol) by the evaluation of the specimens that are sent to Trakya University Hospital Microbiology Laboratory.

Methods: In this study, laboratory data of the cases that were pre-diagnosed with tuberculosis between 11/02/2016 and 31/12/2017 were scanned retrospectively. To compare the annual data descriptive statistics as arithmetic mean, numbers and percentages were used.

Results: Out of 4752 samples, 133 (2.79%) were culture positive. 120 isolates were identified as Mycobacterium tuberculosis complex, while other 13 were defined as Mycobacterium other than tuberculosis. Antimycobacterial susceptibility tests showed that 9 (7.5%) isolates were resistant to streptomycin, 10 (8.33%) isolates to isoniazid, 4 (3.33%) isolates to rifampicin, 4 (3.33) isolates to ethambutol. 5 (4.16%) isolates were multidrug resistant.

Conclusion: Antituberculous drug resistance is still a threat for an effective treatment of tuberculosis and streptomycin resistance has increased. However, it is also pointed out that presence of Mycobacterium other than tuberculosis in isolates has increased.

Keywords: Drug resistance, tuberculosis, streptomycin

INTRODUCTION

Tuberculosis (TB) is an infectious disease that is caused by Mycobacterium tuberculosis complex bacilli which enter the body through the respiratory route and reside in the lungs (1). Atypical isolates such as Mycobacterium other than tuberculosis (MOTT) can also rarely cause the disease. TB is one of the most fatal diseases all around the world (1). Ehrlich-Ziehl-Neelsen (EZN) staining of sputum in order to identify acid-resistant bacteria (ARB), bacterial cultures, clinical history and chest X-ray are utilized for the diagnosis (2, 3). Major anti-TB drugs are streptomycin (SM), isoniazid (INH), rifampin (RIF), ethambutol (EMB) and pyrazinamide (PZA). Other anti-TB drugs are minor ones such as para-aminosalicylic acid, ethionamide, cycloserine, kanamycin, capreomycin, amikacin, thioacetazone, fluoroquinolones (ofloxacin, ciprofloxacin), rifabutin. Major drugs have stronger effects, fewer side

effects than minor drugs. Nevertheless, minor drugs are preferred for multidrug-resistant TB (MDR-TB).

For the treatment, a combination of at least four types of anti-TB drugs, mostly INH, RIF, EMB, SM, and PZA, are used for the first two months (3). This is known as the bactericidal phase which has the maximum amount of bacteria and maximum risk for resistance. Then, maintenance phase follows the bactericidal phase that lasts four or seven months and in this period at least two drugs that contain isoniazid are given to the patient (3). Drug resistance should be monitored before designing the treatment plan if the drug resistance rates for SM, INH, RIF, and EMB in the region are higher than estimated percentages determined by World Health Organization (4). A long period of time is necessary for the treatment and follow-up (1, 3, 4).

In the last twenty years, anti-tuberculosis drug resistance increased significantly all around the world

due to inappropriate use of drugs, insufficient treatment and incomplete adherence (5, 6). Although the incidence of TB decreases over the years, the number of patients infected with resistant strains increases greatly (7). The drug resistance is measured by antimycobacterial susceptibility tests. There are several studies about drug resistance demonstrating the continuous change of the percentages (8, 9). The reason for the multitude of studies concerning drug resistance is that the treatment regimens are developed according to the drug resistance and the problem directly targets the public health.

There are 5 types of drug-resistant TB: Mono-Drug Resistant TB, Poly-Drug Resistant TB, MDR-TB, Extensively Drug Resistant TB and Extremely Drug Resistant TB (10). MDR-TB, a disease originated from *Mycobacterium tuberculosis* complex which is resistant to at least two drugs (especially INH and RIF), is a global threat, as it brings a great challenge in the management of TB. In 2015 there were 4.6% MDR-TB cases among an estimated 10.4 million TB cases worldwide (11). MDR-TB is a crucial problem for the TB control because it completely changes the direction of the treatment. Preventing the development of drug resistance, providing an early detection and proper treatment play an important role to prevent MDR-TB (12).

The aim of this study is to reveal the rates of the *Mycobacterium tuberculosis* complex bacilli resistance to major anti TB drugs (SM, INH, ETB, RIF) by evaluating the specimens that are sent to Trakya University Hospital Microbiology Laboratory in Edirne, Turkey and to compare the annual results.

MATERIAL AND METHODS

This study was approved by Scientific Research Ethics Committee of Trakya University School of Medicine. In this study, laboratory registers of the cases that were pre-diagnosed with tuberculosis between 11/02/2016 and 31/12/2017 were scanned retrospectively. The study was carried out with data from Trakya University Hospital.

Samples of sputum, pleural fluid, urine, peritoneal fluid, tissue, cerebrospinal fluid, bronchial lavage, selective lavage, synovial fluid, endotracheal aspirate, early morning gastric washings and abscess were investigated with EZN staining. Bacterial culture examination and antimycobacterial susceptibility tests were carried

out on BACTEC MGIT 960 (Becton Dickinson Diagnostic, USA) automated system. Tests were performed for the antimycobacterial drugs; SM, INH, RIF, EMB as suggested by National Committee Clinic Laboratory Standards (13). Positive cultures were also identified using the immunochromatographic assay (BD MGIT TBc Identification Test, Becton Dickinson Diagnostic, USA). If there was more than one culture positivity of the same patient, the patient's initial culture was used for drug susceptibility.

Along with the drug susceptibility test results, data including patient's age and obtaining date of the samples were retrieved using laboratory registers.

The results are recorded and grouped according to the year obtained. To compare annual results descriptive statistics as arithmetic mean \pm standard deviation, numbers and percentages were used.

RESULTS

This retrospective study included 4752 different specimens from the patients of Trakya University Hospital. Most of the specimens were sputum (2907) followed by bronchial lavage (628), urine (286) and pleural fluid (174). The mean age of the patients was 49.51 ± 19.40 in 2016 and 53.73 ± 16.54 in 2017. 111 of the specimens were identified as positive in EZN staining and 234 of the specimens were culture positive. These 234 samples belonged to 133 different patients, therefore antimycobacterial susceptibility tests were performed on their initial samples. 133 (2.79%) of these samples showed growth of ARB. *M. tuberculosis* complex was found in 120 isolates, while MOTT was identified in the other 13. Antimycobacterial susceptibility tests showed that 9 (7.5%) isolates were resistant to SM, 10 (8.33%) isolates to INH, 4 (3.33%) isolates to RIF, 4 (3.33%) isolates to EMB. Results also revealed that 5 (4.16%) isolates were multidrug-resistant, as one of them to INH and RIF, one to INH and EMB, two to INH and SM, while another one to all anti TB drugs that we had tested (SM, INH, RIF, EMB). Resistance to SM was 5% in 2016 but was found to be increased to 10% in 2017. On the contrary, INH resistance was 10% in 2016 and it decreased to 6.66% in 2017. Another annual data showed that percentage for MOTT among the isolates were 3.22% in 2016 but it was increased to 15.49% in 2017. Yearly distribution of the results is shown in Table 1.

Table 1: Annual values of the results.

	2016 Number (%)	2017 Number (%)
SM resistance	3 (5)	6 (10)
INH resistance	6 (10)	4 (6.66)
RIF resistance	3 (5)	1 (1.66)
EMB resistance	2 (3.33)	2 (3.33)
INH+RIF resistance	1 (1.66)	0
INH+EMB resistance	1 (1.66)	0
INH+SM resistance	0	2 (3.33)
INH+RIF+EMB+SM resistance	1 (1.66)	0
Total MDR-TB	3 (5)	2 (3.33)
<i>M.tuberculosis</i> complex	60 (96.78)	60 (84.51)
MOTT	2 (3.22)	11 (15.49)
Total isolates	62	71

INH: Isoniazid, RIF: Rifampin, EMB: Ethambutol, SM: Streptomycin, PIR: Pyrazinamide, MDR-TB: Multidrug-Resistant Tuberculosis, MOTT: Mycobacterium Other Than Tuberculosis

DISCUSSION

Tuberculosis is one of the most important chronic infectious diseases with its high global rates of morbidity and mortality, especially in developing countries such as Turkey (14). Therefore, preventing and therapeutic strategies against this disease are of crucial importance especially in countries where tuberculosis is endemic. Early diagnosis, proper and regular treatment of patients and patient follow-ups after treatment are essential components of tuberculosis control programs (15). Evaluation of the success of this treatment strategies can be made by analyzing the epidemiological data (7, 9).

Limited availability of antituberculosis drugs and the development of resistance against these drugs are not only public health concerns for developing countries, but also for the developed countries (8, 16, 17). Among the causes of resistance development, the most important ones are noncompliance to medication and misapplication strategies. Also, inadequate drug dosing and/or absorption contribute to the resistance development (8, 14, 17, 18). The incidence of tuberculosis decreases, whereas the rate of resistance increases (7).

In our study, SM (7.5%) and INH (8.33%) resistances showed an increase compared to other anti-TB drugs. Previous studies in Edirne identified the resistance rates as 8.1%, 9.7% and 8.2% for SM, 11.5%, 12.6% and 14.4% for INH in 2005, 2006 and 2007, respectively. They also revealed the MDR as 6% (13). Percentages for INH resistance (8.33%) and MDR (4.16%) decreased in our study. Another study on anti TB drug

resistance was carried out by Tansel et al. (9) on 139 isolates between 1999 and 2001 in Edirne. Findings of the study for INH, RIF, and EMB were similar to our study. Although the percentages were consistent with our study, resistance for SM (2.2%) was quite lower at that time. Comparison with this study indicates an increase in SM resistance.

Another study carried out in Sivas revealed that resistance to RIF (10.4%) and EMB (9.1%) were higher than INH (5.2%) and SM (2.6%) (19). MDR's value also surpassed the value in our study (4.16%) with 14.3%. In our study, resistance to RIF (3.33%) and to ETH (3.33%) were lower. On the other hand, resistance to SM (7.5%) was higher.

For the surveillance in Ankara, another study was carried out by Sezen et al. (20). They detected the resistance to INH as 3,8%, to RIF 0,9%, to SM 3,8%, EMB to 0,5% (20). All of the resistance rates were lower than those in our study. Considering these results, the location may have a role in drug resistance.

This was a retrospective study carried out using data drawn from the laboratory registers, however, patient files were not used during the study. This was a limitation of our study.

In conclusion, this study revealed that anti TB drug resistance remains a problem for the proper treatment of tuberculosis and resistance to SM has increased. Another aspect to point out is that the presence of MOTT in the isolates has increased over the years. Therefore, follow-up studies are needed to be carried out.

Ethics Committee Approval: This study was approved by Scientific Researches Committee of Trakya University School of Medicine.

Informed Consent: Written informed consent was obtained from the participants of this study.

Conflict of Interest: The authors declared no conflict of interest.

Author contributions: Concept: OÖ, CE. Design: OÖ, İEÖ, KE, CE. Supervision: OÖ, İEÖ, KE, CE. Resources: OÖ, İEÖ, KE, CE. Materials: OÖ, İEÖ, KE, CE. Data collection and/or processing: OÖ, İEÖ, KE, CE. Analysis and/or Interpretation: OÖ, İEÖ, KE, CE. Literature Search: OÖ, İEÖ, KE, CE. Writing Manuscript: OÖ, İEÖ, KE, CE. Critical Review: OÖ, İEÖ, KE, CE.

Financial disclosure: The authors declared that this study received no financial support.

Editor-in-chief's Note: Two of the authors of this article, Ozan Öner and Kubilay Elmacı are members of the editorial board of Turkish Medical Student Journal. However, they did not take place in any stage on the editorial decision of the manuscript. The editors who evaluated this manuscript are from another institutions.

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