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Drug Resistance Tests for Mycobacterium Tuberculosis and Compatibility between Tests; From Past to Today

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ABSTRACT

Objective: The gold standard for pulmonary tuberculosis diagnosis is the demonstration of Mycobacterium tuberculosis bacilli. Drug susceptibility test results of the bacilli obtained are crucial in tuberculosis treatment management. The various tests used to determine drug susceptibility must yield the same result. In the present study, we aimed to evaluate the compatibility of drug susceptibility test results obtained by Löwenstein-Jensen (L-J) and BACTEC 460TB methods from the past to the present.

Material and Methods: Sputum results from 79 patients suspected of multidrug-resistant pulmonary tuberculosis (MDR-TB) clinically, radiologically, and bacteriologically were evaluated for isoniazid, rifampicin, ethambutol, and streptomycin between June 1997 and June 1998. Culture and drug sensitivity tests on the L-J medium were conducted at the Heybeliada Chest Diseases and Thoracic Surgery Center bacteriology laboratory, while culture and drug sensitivity tests with the BACTEC 460 TB system were performed at another center. The results were assessed for compatibility using the Kappa (κ) test, a tool for comparing two independent parameters.

Results: All drug sensitivity tests for isoniazid, rifampicin, ethambutol, and streptomycin were collectively evaluated. It was determined that 263 (83.3%) of 316 drug sensitivity tests yielded concordant results, while 53 (16.7%) produced discordant results. The drug sensitivity tests using L-J and BACTEC 460TB methods indicated compatibility only for streptomycin (κ = 0.715). In contrast, they yielded different results for isoniazid (κ = 0.585), ethambutol (κ = 0.552), and rifampicin (κ = 0.507). Streptomycin exhibited compatibility, while isoniazid, ethambutol, and rifampicin showed incompatibility between the L-J and BACTEC 460TB methods.

Conclusion: Drug sensitivity tests are pivotal in tuberculosis treatment management. While literature suggests compatibility between L-J and BACTEC 460TB methods, our study revealed incompatibility. Evaluation of drug sensitivities may lead to confusing results. Current practices involving studies in the same laboratory and genetic testing contribute to faster and more accurate outcomes in managing drug-resistant tuberculosis. Genetic tests and reference laboratories remain crucial for antituberculosis drug sensitivity, emphasizing their continued importance.

Keywords: Tuberculosis, Multi-drug resistance, Drug, Resistance

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INTRODUCTION

Tuberculosis (TB) is an infectious disease attributed to a group of Mycobacteria collectively known as the Mycobacterium tuberculosis complex. The most common form of TB is pulmonary tuberculosis. The global burden of tuberculosis and the difficulties encountered in its diagnosis and treatment, especially in low- and middle-income countries. It is a serious public health issue, as it is transmitted through the respiratory tract. The fight against tuberculosis continues in our country and globally under the End TB strategy program of the World Health Organization (WHO) (1, 2).

It is currently estimated that there are 10 million tuberculosis patients in the world, 500 thousands of them have drug-resistant tuberculosis, and only 1/3 of the resistant patients can be treated appropriately. Three million were bacteriologically confirmed, and only 2.1 million were tested for rifampicin resistance (3).

The primary strategy in tuberculosis treatment revolves around the prompt identification of the bacillus and the initiation of treatment. Guidelines advocate for the demonstration of bacilli in biological materials, such as sputum, and the assessment of drug resistance when deemed necessary (1). Drug resistance should be considered in previously treated cases (drug resistance is highest in cases of treatment failure, relapses, and cases who return after being lost to follow-up are also referred with suspicion of resistance/fast rifampicin resistance studies), patients who have been in places with high drug resistance (especially in the former Soviet Union countries), patients with a history of contact with resistant TB patients, patients who have received irregular treatment, patients with a history of contact with a previously treated patient, patients who are smear positive at the end of the third month of treatment, and patients who are not recovered over the course of treatment (4). However, the long duration of diagnosis and antibiotic susceptibility testing for Mycobacterium tuberculosis (M. Tuberculosis) affects the success rate in treating and controlling the disease. In 2021, 120 cases of rifampicin resistance (RR), 101 cases of resistance to any quinolone, 10 cases of pre-extensive drug resistance (cases with any quinolone resistance in addition to isoniazid and rifampicin resistance (pre-XRR)), and 9 cases with resistance to any other drug were reported from Turkey (5). Globally, 1.5 million rifampicin-resistant/ MDR-TB patients were planned to be treated between 2018 and 2022, whereas 649,000 patients (48% of the target) were treated (2). The time for obtaining drug resistance test results may vary depending on the tests used. There is a range of methods for drug susceptibility testing, and various tests can be performed in different institutions. Occasionally, it may be observed that these tests do not give compatible results in terms of drug susceptibility results. The present study compares in vitro drug sensitivity tests performed on the Löwenstein-Jensen (L-J) medium and BACTEC method and discusses the current status of drug resistance testing.

MATERIAL and METHODs

Patients with clinical, radiological, and bacteriological suspicion of multidrug-resistant tuberculosis (MDR-TB) who were admitted to Heybeliada Chest Diseases and Thoracic Surgery Center between June 1997 and June 1998 were included in the study. The patients were over 18 years of age. Pulmonary tuberculosis was demonstrated clinically, radiologically and by the presence of bacilli in their sputum. Culture and drug sensitivity tests on the Löwenstein-Jensen medium were performed at the Heybeliada Chest Diseases and Thoracic Surgery Center bacteriology laboratory, while culture and drug sensitivity tests with the BACTEC 460TB system were performed at another center. Sputum samples of 79 patients were collected with a maximum interval of 1 day between two samples and evaluated for sensitivity to four drugs (isoniazid, rifampicin, ethambutol, streptomycin) using two methods.

Absolute concentration method in Löwenstein-Jensen medium

If the sputum preparation is positive for bacilli, the sputum is homogenized. The top of the tube is poured, leaving 1 ml of sputum at the bottom. The residual precipitate is inoculated with 0.1 ml of sputum samples into L-J media, both with and without drugs (control). If bacilli cannot be detected in direct preparations, bacilli are produced in the medium first, and then drug susceptibility tests are performed for resistance. Resistance assessment is performed after approximately 1 month in an oven at 37 C. Growth in the control tube and in the medicated tubes are evaluated. Reproductive intensity is indicated by the number of colonies up to 10 colonies, between 10 and 100 colonies (+), more than 100 colonies but countable (+++), and more than 100 colonies but too many to count (++++). It was considered resistant if the number of colonies in the medicated tubes in which growth was determined was above 10. Drug concentrations used for drug sensitivity in the L-J medium and BACTEC test are shown in **Table 1**.

Table 1: Drug concentrations used in drug sensitivity tests conducted using the Löwenstein-Jensen and BACTEC methods.

Drug	Concentration Löwenstein-Jensen	Concentration BACTEC
Isoniazid	1.0 µg/ml	0.1 µg/ml
Streptomycin	10 µg/ml	2.0 µg/ml
Rifampicin	40 µg/ml	2.0 µg/ml
Ethambutol	2.0 µg/ml	2.5 µg/ml

BACTEC TB 460 Susceptibility Test

In the BACTEC method, the growth on the medium was assessed using a BACTEC 460 semi-automatic device. It was tested every 2-3 days for the first 2-3 weeks and then once a week for a total of 6 weeks. When the growth index (GI) was 50-100, a smear was taken and stained with the Ziehl-Neelsen method and reported as positive culture if it was found positive. Drug susceptibility testing using the BACTEC method is quite similar to the primary isolation procedure. When an antituberculosis drug is added to the medium, growth is suppressed if the bacilli are susceptible. This suppression may be identified by observing a decrease or a minimal increase in daily gastrointestinal (GI) activity compared to the control.

If the bacillus is resistant, minimal to no suppression is observed. To ascertain a 1% resistance, the quantity of bacteria utilized in the control vial was 100 times less than in the drug-containing vial. Both drug and control vials underwent daily testing after inoculation. Bacilli were classified as drug-resistant if the rate of increase in gastrointestinal (GI) activity or the change in amount compared to the previous day (ΔGI) was equal to or greater than that of the control vial. Conversely, for a susceptible population, the daily increase in the control vial would surpass that of the test vial.

In the laboratory, nicotinamide was used in drug susceptibility tests, which was thought to have the same activity in vitro due to the chemical properties of pyrazinamide (because the medium was basic). Therefore, pyrazinamide was not included in the study.

The results were compared in terms of compatibility with the Kappa (κ) test, which is used to compare two independent parameters, and the results were evaluated as incompatible when the κ value was <0.70 and compatible when the κ> value was 0.70.

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RESULTs

Out of 316 drug susceptibility tests for the four drugs, 263 (83.3%) yielded the same result, and 53 (16.7%) yielded different results with the L-J and BACTEC methods. The results obtained for all drugs are shown in **Table 2**.

Drug resistance compatibility of both methods was found to be incompatible for isoniazid, rifampicin, and Ethambutol (κ= 0.585, κ = 0.507, and κ=0.552, respectively). For streptomycin, the two tests showed compatible results (κ= 0.715).

Table 2: Results obtained for drugs.

			BACTEC method n (%)			
			S	R	T	Statistics *
The Löwenstein-Jensen Method n (%)	**Isoniazid**	S	15 (19.0%)	8 10.1%)	23 (29.1%)	**κ <0.70** (κ= 0.585)
		R	5 (6.3%)	51 (64.6%)	56 (70.9%)	
		T	20 (25.3%)	59 (74.7%)	79 (100%)	
	Rifampicin	S	10 (12.65%)	3 (3.8%)	13 (16.5%)	**κ <0.70** (K= 0.507)
		R	10 (12.65%)	56 (70.9%)	66 (83.5%)	
		T	20 (25.3%)	59 (74.7%)	79 (100%)	
	Ethambutol	S	33 (41.8%)	15 (19%)	48 (60.8%)	**κ <0.70** (κ= 0.552)
		R	3 (3.8%)	28 (35.4%)	31(39.2%)	
		T	36 (45.6%)	43 (54.4%)	79 (100%)	
	Streptomycin	S	53 (67.1%)	1 (1.3%)	54 (68.4%)	**κ>0.70** (κ= 0.715)
		R	8 (10.1%)	17 (21.5%)	25 (31.6%)	
		T	61 (77.2%)	18 (22.7%)	79 (100%)	

DISCUSSION

At the time of this investigation, the present study marked the first instance in Turkey where L-J and BACTEC drug tests were compared concerning drug resistance test results. The findings indicated that while L-J and BACTEC tests produced concordant results for streptomycin, they diverged for isoniazid, ethambutol, and rifampicin in terms of drug resistance outcomes. It's noteworthy that the BACTEC method and the L-J method were conducted in separate laboratories during the relevant period, implying that different sputum samples from the same patient could be analyzed.

A comparison of drug susceptibility tests previously performed with the conventional method and the BACTEC 460TB system showed 98% compatibility. Only 8 out of 424 drug susceptibility tests showed incompatible results, most of which were for streptomycin (6). Our study, in contrast, showed compatible results only for streptomycin.

In a later study, the proportion method was compared between the BACTEC 460 TB system and commercially available L-J medium with ready-to-use antibiotics. The resistance rates of 238 M. tuberculosis strains to streptomycin, isoniazid, rifampicin, and ethambutol were 19.7%, 42%, 40.8%, and 18%, respectively, using the BACTEC 460 TB system and 22.7%, 38.7%, 37%, and 15.5%, respectively, using the L-J proportion method. No statistically significant difference was found between both methods in terms of resistance rates (p> 0.05) (7). In the current study, although drug resistance rates were numerically distinct, no statistically significant difference was identified.

Aktas et al. compared four different susceptibility tests for first-line drugs used to treat Mycobacterium tuberculosis (8). BACTEC 460 TB, manual Mycobacteria Growth Indicator Tube (MGIT), and L-J proportion methods were compared for 50 M. Tuberculosis complex strains using the agar proportion method, considered as the standard reference method.

The BACTEC 460TB and agar proportion methods were 100% compatible with all four drugs. Compatibility with the MGIT method was 98% for isoniazid and ethambutol and 100% for streptomycin and rifampicin. A strain found to be resistant to ethambutol and isoniazid via the agar proportion method was found to be sensitive via the MGIT method. In this study, the compatibility between the L-J and the agar proportion methods was 94% for ethambutol and 90% for isoniazid, rifampicin, and streptomycin. The turnaround times for agar proportion, L-J, BACTEC 460TB, and MGIT methods were 20.2 days, 28 days, 5.8 days, and 7.1 days, respectively. The fastest and agar proportionally compatible result was obtained from the BACTEC 460TB system (8). The concentration of isoniazid used in L-J in this study was lower than that used in our study (0.2 µg/ml vs 1.0 µg/ml). This difference may be a factor affecting our study's incompatible results for isoniazid.

In another study comparing the radiometric BACTEC TB460 and BACTEC MGIT 960 tests for drug resistance for four drugs, the overall compatibility between the two tests was 96.7% (9). In this study, the BACTEC TB460 system showed low specificity for ethambutol, while the BACTEC MGIT 960 system showed higher specificity and sensitivity. However, the BACTEC 460TB system provided results 2.5 days faster (9).

In a study reporting the results in Canakkale province between 2009-2011, growth was detected in 78 (7.44%) and 68 (6.48%) of 1048 samples using BACTEC MGIT 960 and L-J methods, respectively. However, the results of 25 patients whose drug resistance results were analyzed were not compared in terms of compatibility between the two methods (10).

Compatible results were reported in drug susceptibility tests for TB using various methods in the literature. The major limitation in the difference in the results of our study could be that the tests were performed on sputum obtained from the

same patient but at different times due to an intervening day. Even if the sputum belongs to the same patient, the bacillus populations in the sample may vary, leading to different results.

Today, the Xpert MTB/RIF test can provide results in 1 hour and 45 minutes to detect rifampicin resistance. "Line probe" tests can be utilized for isoniazid and rifampicin resistance or quinolone and parenteral drug resistance. The results can be obtained within 1-2 days (4).

CONCLUSION

The results of drug susceptibility tests were not compatible between L-J and BACTEC 460TB systems (except streptomycin) in our study. However, studies are showing that the results of tests performed in ideal laboratory environments on appropriate samples are compatible. Furthermore, genetic tests, which provide results in a very short time, have an essential role in guiding treatment and the treatment of tuberculosis.

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Ethical approval: The present study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki. Ethical approval for the study was obtained from the appropriate ethics committee..

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