INVESTIGATION OF THE SUSCEPTIBILITIES OF MYCOBACTERIUM TUBERCULOSIS COMPLEX STRAINS TO MAJOR ANTITUBERCULOSIS DRUGS WITH BACTEC MGIT 960 SYSTEM

Muhammet Güzel Kurtoğlu MD,[1] Recep Kesli MD,[1] Yüksel Terzi Assist. Prof. MD,[2] Mahmut Baykan Prof. MD[3]

[1] Konya Educational and Research Hospital, Microbiology and Clinical Microbiology Laboratory, Konya.

[2] Ondokuz Mayıs University, Faculty of Art and Science, Department of Statistics, Samsun.

[3] Selcuk University Faculty of Medicine, Department of Microbiology and Clinical Microbiology, Konya

ABSTRACT

Objective: *The study was designed to investigate retrospectively the resistance rates of tuberculosis-causing mycobacteria, isolated in the microbiology and clinical microbiology laboratories of Konya Research and Education Hospital.*

Material and Method: *Mycobacterium tuberculosis complex strains were isolated from various clinical samples of 1666 patients applying to Konya Research and Education Hospital between May 2007 and December 2009, and the resistance rates of Mycobacterium tuberculosis complex strains against first generation anti tuberculosis drugs were investigated. After homogenization and decontamination, the samples investigated were cultured using BACTEC Mycobacteria Growth Indicator Tube-960 (MGIT-960) system. Susceptibility rates of the strains determined with production were investigated with the same system versus streptomycin (SM), Isoniazid (INH), Rifampin (RIF) and Ethambutol (ETB) (SIRE).*

Results: *From 1666 patients prediagnosed with tuberculosis, 70 Mycobacterium tuberculosis complex strains were isolated. While no drug resistance was found in 17 (24%) of them, resistance to one or two drugs was found in 26 (37%) strains (24% to INH, 20% to SM, 6% to ETB and 4% to RIF). While resistance was found to be only against one drugs in 15 of these (21%), two drugs in 11 of these (16%), no resistant strains could be determined against three or four drugs together. Among the patients with resistance, 81% (57/70) displayed primary and 18% (13/70) secondary tuberculosis, and 2 patients were found to display resistance to isoniazid and rifampin together (MDR-TB).*

Conclusion: *It was seen that the findings in the study were consistent with those determined by other studies in our country. Preventing the resistance to antituberculosis drugs is possible by enlightening the distribution rates of drug resistant strains in public, defining appropriate drug regimes and increasing the quality of tuberculosis control programs. Therefore, it is essential that regular and continuous scanning of antituberculosis drugs should be performed.*

Key Words: *Mycobacterium tuberculosis complex, drug resistance, tuberculosis, multi-drug resistance.* ***Nobel Med 2011; 7(1): 42-48***

MYCOBACTERİUM TUBERCULOSİS KOMPLEKS SUŞLARININ MAJOR ANTİTÜBERKÜLOZ İLAÇ DUYARLILIKLARININ BACTEC MGIT 960 SİSTEMİYLE ARAŞTIRILMASI

ÖZET

Amaç: Çalışma, Konya Eğitim ve Araştırma Hastanesi Mikrobiyoloji ve Klinik Mikrobiyoloji Laboratuvarı'nda üretilen tüberküloz etkeni mikobakterilerin antitüberküloz ilaçlara karşı direnç oranlarının retrospektif olarak araştırılması amacıyla planlanmıştır.

Materyal ve Metod: Mayıs 2007 ile Aralık 2009 tarihleri arasında Konya Eğitim ve Araştırma Hastanesine tüberküloz ön tanısıyla başvuran 1666 hastanın çeşitli klinik örneklerinden üretilen Mycobacterium tuberculosis kompleks suşlarının birinci kuşak anti tüberküloz ilaçlara karşı direnç oranları araştırılmıştır. İncelemeye alınan örnekler BACTEC Mycobacteria Growth Indicator Tube-960 (MGIT-960) sisteminde kültürleri yapılmıştır. Üreme saptananların aynı sistemle duyarlılık oranları incelenmiştir.

Bulgular: Tüberküloz ön tanısıyla başvuran 1666 hastadan 70 Mycobacterium tuberculosis kompleks suşu izole edilmiştir. Bu suşların 44'ünde (%63) ilaç direncine rastlanmazken; 26 (%37) suşta bir veya birden fazla ilaca direnç (isoniazid'e %24 (17/70), etambutol'e %6 (4/70), streptomisin'e %20 (14/70), rifampin'e %4 (3/70)) bulunmuştur. Onbeş suşta (%21) bir ilaca, 11 suşta (%16) iki ilaca direnç saptanırken, 3 veya 4 ilaca birden dirençli suş saptanmamıştır. Primer tüberküloz %81 (57/70), sekonder tüberküloz ise %18 (13/70) olarak saptanmış olup sadece 2 olguda isoniazid ve rifampine birden direnç tespit edilmiştir (ÇID-TB).

Sonuç: Toplumlardaki ilaca dirençli suşların dağılımlarını ortaya koymak, uygun ilaç rejimlerini belirlemek ve tüberküloz kontrol programlarının kalitesini değerlendirebilmek için anti tüberküloz ilaç direnç taramaları düzenli ve sürekli olarak yapılmalıdır.

Anahtar Kelimeler: Mycobacterium tuberculosis complex, ilaç direnci, çoğul ilaç dirençli tüberküloz. **Nobel Med 2011; 7(1): 42-48**

INTRODUCTION

Among Mycobacteria, there exist many pathogens and subprofit species, and *M.tuberculosis*, as a significant human pathogen, still remains to be a considerable one.[1-4] A third of the individuals has been estimated to be infected with tuberculosis bacillius worldwide. More than 80 percent of the tuberculosis patients have been witnessed in the form of lung tuberculosis.[5] Almost 2 million people per annum and nearly one individual in every other 17 seconds have been dying as a result of tuberculosis. Ninety-eight percent of the deaths stemming from the disease have taken place in developing countries. Turkey is among the countries where incidence rates of tuberculosis are encountered in the middle category.[6]

Due to the discovery of effective novel drugs used in the treatment of tuberculosis in the second half of 20th century, a significant and continuous decrement was witnessed in the morbidity and mortality rates of tuberculosis in industrialized countries. However, the decrement witnessed in the incidence of tuberculosis ceased after mid-1980s, and even an increase of 12% was witnessed in the number of the patients between 1985 and 1995. As the reason of the increase, the insufficient eradication programmes and the expansion of HIV are considered to be responsible.[7-9] The most significant challenge for tuberculosis is now the increase in the patients of multidrug resistant (MDR) tuberculosis. The increase in the patients of HIV, as well as tuberculosis, makes the challenge more critical. In the light of the literature, resistance has commonly been observed against INH and RIF, the most considerable antituberculosis drugs, and it was reported that resistance could also be able to develop against other antituberculosis drugs. It is essential that the patients be defined as soon as possible in order to prevent MDR tuberculosis and the treatment be administered without delay.[7,10] No matter how there unexists a detailed nationwide study to show the resistance profile in Turkey, different results have been found in various local studies. Primary and secondary resistant rates were reported to change from 18-27% to 28-53% against at least one major drug, respectively.[6,11] According to the third "WHO/IUATLD Global project on Anti-tuberculosis Drug" report, the average primary and secondary resistance rates were determined as 10.2% and 18.4% against at least one drug woldwide, respectively.[6]

In our study, it was aimed to investigate the resistance rates of tuberculosis-causing mycobacteria to major anti-tuberculosis drugs, isolated from the patients applied to our department with the prediagnosis of tuberculosis.

MATERIAL and METHOD

In this study, *M.tuberculosis* complex strains isolated→

from the clinical samples of 1666 patients, applied to Konya Education and Research Hospital between May 2007 and December 2009, with the prediagnosis of tuberculosis, were investigated.

Preparation of the Samples: After the homo genization and decontamination of sputums, gastric aspirates, bronchial lavages, urines, purulent and other mucopurulent materials; sterile bodily fluids, such as cerebro-spinal fluid (CSF), synovial fluid and pleural fluid, were directly inocculated without decontamination. For the homogenization and decontamination procedures of the samples, N-acetyl-L-systein - 4% of NaOH - 2.9% of sodium nitrate (NALC-NaOH) method was used. An amount of 5-10 ml from each sample was mixed with NALC-NaOH solution, at equal amount, being poured into the tubes, and then the mixture was vortexed without exceeding 30 sec. The tubes were kept at room temperature for 15 min. by turning the tubes upside down by hand from time to time. Phosphate buffer (0.067 M, pH= 6.8) was added onto the mixture into each tube up to 50 ml and centrifuged at 3000Xg for 15 min. The sedimentations provided were diluted with 1-2 ml of phosphate buffer (PH=6.8). Preparates were prepared from the solutions in order to use for dying and were innoculated into Bactec Mycobacterium Growth Indicator Tube (MGIT) 960 culture media (BD, Biosciences, Sparks, MD, Ireland).

Isolation of Mycobacteria: Prior to the inoculation, oleic acid-albumin- dextrose- catalase (OAOC) was added into the culture media as a concentrator. As an antimicrobial agent, an antibiotic mixture (PANTA, Becton Dickinson, Sparks, MD, USA), including polymyxin B (50 U/mL), azlocillin (10 mcg/mL), nalidixic acid (20 mcg/mL), trimethoprim (5.0 mcg/mL) and amphotericin B (5.0 mcg/ml) was added. Samples of 0.5 mL obtained from the prepared clinical mixtures were inocculated into Bactec Mycobacterium Growth Indicator Tube (MGIT-960, BD, Biosciences, Sparks, MD, Ireland) culture media and kept in BACTEC MGIT-960 device at 37°C for 6-8 weeks. For the samples determined with no production during this period, the findings were reported to be negative.

Definition of *M.tuberculosis*: Having been performed a passage onto Colombia agar culture media (Becton Dickinson-Germany) including 5% of sheep blood in the positive tubes when noticed production signals, the production was investigated to find out whether to arise from the contamination or not. In addition, positive MGIT tubes determined to be with production were investigated as for the existence of ARB and the feature of forming cord by preparing preparates with EZN dyes from the positive tubes. Performing an additional identification test, in the culture media BACTEC NAP (r-nitro-α-acetylamino-β-hydroxypropiophenone, Becton Dickinson, Sparks, MD, USA) in the positive culture media, the separation of *M.tuberculosis* complex strains and Mycobacteria other than tuberculosis (MOTT) was carried out. In the evaluation of the results, bacilli were considered to be M.tuberculosis complex if Growth Index (GI) in NAP test bottle decreased or unchanged while GI in control bottle increased. On condition that an increase was observed in GI of NAP test bottle, bacilli were determined as MOTT.

Susceptibility Tests: BACTEC-960 SIRE kits were used to investigate the susceptibility rates of the strains defined as *M.tuberculosis* complex via NAP test to major antituberculosis drugs [SM (2.0 µg/mL), INH (0.1 µg/mL), RIF (2.0 µg/mL), ETB (2.5 µg/mL) = SIRE]. Those found to be resistant to INH and RIF were termed as Multidrug Resistant Tuberculosis (MDR-TB). Upon retrospectively investigating the records of the patients, primary and secondary resistance rates were evaluated, classifying the patients as novel patients, unsuccessful treatment, those discontinuing treatment protocole and those appropriate for the definition of recurrence under the criteria formed by World Health Organization (WHO)[12]. The study protocole was approved by the local ethic committee, under the Declaration of Helsinki. For statistical analysis, two-proportion test was performed, and $p<0.05$ was accepted to be significant.

Ethical Approval: Ethical approval was provided from Ethics Comittee of Meram Medical School, Selçuk University, Konya, Turkey.

RESULTS

Of total 1666 patients applied to our department between May 2007 and December 2009 with the prediagnosis of tuberculosis, 695 women (42%) and 971 men (58%) were enrolled into the study. Mean age rate was 55.17±19.04 (for women, 54.09±19.76; for men, 55.96±18.48). Resistance in the patients without previous tuberculosis history was accepted as primary, and those with previous tuberculosis history (recurrence, unsuccessful treatment and those discontinueing the treatment) were accepted to be with secondary resistance.

Among 78 patients ordered with culture samples and determinated to be ARB positive, 48 (61.5%) were men and 30 (38.5%) were women. Cultures of 78 patients with ARB positivity were inocculated, and production was observed in 70 (89.7 %) of them. Of 70 patients found to be positive, 44 (63%) were men →

and 26 (37%) women, and mean age rate was 50.5 (for women, mean age rate was 59 and for men 43). A significant difference was found between the mean age rates of women and men (P=0.03<0.05). Merely a sample from one patient was evaluated in the same year. Sixty four of the materials (92%) found to be positive were sputum originated, 3 (4 %) pleural fluid and 1 (1%) CSF (Table 1). Ninety-two percent of the patients were lung tuberculosis.

Within 70 *M.tuberculosis* complex strains isolated, while no resistance was encountered in 44, resistance rates to one or two drugs were determined in 26 (37%) (to INH 24%, to ETB 6%, to SM 20% and to RIF 4%). While resistance was determined to drugs together in 11(16%) of them (1 strains to INH and ETB, 2 strains to INH and RIF, 2 strains to SM and ETB, 4 strains to INH and SM and 2 strains also to SM and RIF), no resistant strains to 3 or 4 drugs together were determined. Primary tuberculosis was detected in 57 (81%) of the patients diagnosed with production, and secondary tuberculosis in 13 (18%). While no MDR-TB was found in primary resistant subjects, MDR-TB was found to be resistant to INH and RIF together in only 2 strain (2/13) in secondary resistant subjects. In our study, however, MDR-TB rate was found to be 8% (2/26) among the subjects with resistance and to be 3% (2/70) among all the subjects diagnosed with tuberculosis. Among the patients diagnosed with lung tuberculosis, total resistance rates for INH, RIF, SM and ETB were also found to be 25% (16/64), 5% (3/64), 19% (12/64) and 5% (3/64), respectively. Due to the restricted number of the strains determined in our study, a part of the values was only reported as numerical value, not percentile. Anti-tuberculosis drug resistance rates in all subjects were presented in Table 2.

Table 1: Culture and ARB positivity rates of samples.

Sample	Culture (+)		ARB (+)	
	N	%	n	%
Sputum	64	92	33	94
Pleural fluid	3	4	1	3
CSF	1	1	0	0
Urine	2	3	1	3
Total	70	100	35	100

Table 2: Anti-tuberculosis drug resistance rates in all subjects. N (%)

Drugs (µg/mL)	Primary resistance 17 (%24)	Secondary resistance 9 (%13)	*Total (n:70)
INH (0.1)	7	10	17 (%24)
SM (2.0)	7	7	14 (%20)
ETB (2.5)	1	3	4 (%6)
RİF (2.0)	0	3	3 (%4)
MDR-TB	0	2 (%3)	2 (%3)

*Values in this column are the resistance rates determined from all subjects diagnosed with tuberculosis without any primary and secondary resistance discrimination.

Statistical analysis

Chi-square (χ^2) and Mc-Nemar tests were used for statistical analysis by the programme SPSS 15.0. Differences between gender and positive culture have been researched. The null hypothesis and the alternative hypothesis are as follows:

H_0= There is no difference between gender and positive culture.

H_1= There is a difference between gender and positive culture.

The result of the analysis shows no significant relationship between gender and positive culture (p> 0.05). Thus H_0 is not rejected.

Differences between age and positive culture have been researched. The null hypothesis and the alternative hypothesis are as follows:

H_0= There is no difference between age and positive culture.

H_1= There is a difference between age and positive culture.

The result of the analysis shows no significant relationship between age and positive culture (p> 0.05). Thus H_0 is not rejected.

Differences between sample and positive culture have been researched. The null hypothesis and the alternative hypothesis are as follows:

H_0= There is no difference between sample and positive culture.

H_1= There is a difference between sample and positive culture.

The result of the analysis shows a significant relationship between sample and positive culture (p< 0.05). Therefore H_0 is rejected and H_1 is accepted.

Differences between ARB and positive culture have been researched. The null hypothesis and the alternative hypothesis are as follows:

H_0= There is no difference between ARB and positive culture.

H_1= There is a difference between ARB and positive culture.

The result of the analysis shows a significant relationship between ARB and positive culture (p< 0.05). Therefore H_0 is rejected and H_1 is accepted.

DISCUSSION

After antimicrobial drugs had become available, great advances took place in the course of tuberculosis. →

Table 3: Metaanalysis Results between 1984-2004.[16,17,19,20]

	Primary resistance (%)			Secondary resistance (%)			*Total resistance (%)		
Drugs	1984-89	1990-95	1995-2004	1984-89	1990-95	1995-2004	1984-89	1990-95	1995-2004
INH	14.4	8.8	14.4	34.1	30.1	34.1	27.8	23.8	27.8
SM	8.8	10.1	8.8	24.6	17.7	24.6	22.5	17.9	22.5
ETB	2.2	3.0	2.2	13.3	13.7	13.3	7.8	7.7	7.8
RIF	5.7	8.9	5.7	23.1	31.9	23.1	22.3	22.1	22.3
TOTAL	26.1		26.1	45.8		45.8	40.6		40.6

*These are the resistance rates determined in all patients with tuberculosis without any primary and secondary resistance differentation.

Treatment of TB with medications is the basic of the disease control, and it was observed that treatment rate is quite high, and the contamination risk is eliminated when patients are administered accurate treatment regimes. Drug resistance was observed shortly after first discovered antimicrobial drugs came into use. Drug resistance in the treatment of TB is a challenge coming to the fore with the use of antibiotic drugs and keeping on increasing today. Moreover, the increase in drug resistance leads to serious problems in morbidity and mortality rates, unsuccessful treatment and cost-effectiveness.[13,14]

A third of world population is estimated to be infected with tuberculosis bacilli. Turkey is among the countries where the incidence of tuberculosis is at medium level. The number of the patients with tuberculosis who were reported to WHO was 18043 in 2002, and incidence rate was 0.0026%.[6] As years went by, incidence rates of TB have decreased in Turkey, and the deaths originating from TB decreased from 0.0262% to 0,001.6%.[6] Despite contemporary diagnosis, treatment and control options, tuberculosis still remains a significant public health threat both in Turkey and worldwide. Therefore, the approach to the patients with TB should be standardized. It is essential that susceptibility tests should markedly be performed on a regular basis in the prediagnosis and follow-up periods.

Tuberculosis drug resistance is a significant sign that the regime applied to control tuberculosis has showed little or no effects so far, and the system is inadequate. In the treatment of tuberculosis, a gradual increase in the drug resistance is the most significant handicap preventing the success of the treatment. Main causes in unsuccessful treatment are classified in the literature as the procedures of inadequate treatment regimes, irregular intake of the drugs, early discontinuation of the treatment, drug toxicity and initial resistance.[15]
In the light of the literature published in Turkey, relatively high rates of resistance are reported against tuberculosis drugs.[16-18] In our study, the highest resistance rates in primary and secondary patients were determined against INH and SM. Compared to the findings determined in other metaanalysis studies performed between 1984-1989, 1990-1995 and 1995-2004, the primary and secondary resistance rates (Table 3) were found to be highest against INH and SM, as in our study.[16,17,19,20]

In the analysis of the studies performed in tuberculosis control dispensaries and other different health centers in Turkey, the resistance rates to at least one drug were reported to be between 28 and 53.4% in tuberculosis bacilli isolated from newly diagnosed patients.[6,11] In our study, the resistance rate to at least one drug was found as 37% (26/70), and this rate is consistent with those obtained in other studies.[6,11]

In the study performed by Dogan et al. in Sivas, a province in Central Turkey, primary resistance rates were found to be 37.3% in new cases; primary resistance rates were reported to be 19.9% for INH, 4.1% for RIF, 5.2% for SM and 2.5% for ETB. Secondary rates were also reported to be 53.6% in the study, and the rates were determined to be 26.1% for INH, 10.1 % for RIF, 5.8% for SM and 4.3% for ETB. In the same study, primary resistance rates in new subjects with lung tuberculosis were reported to be 19.7% for INH, 3.8% for RIF, 4.1% for SM and 2.6% for ETB, and secondary resistance rates to be 24.2% for INH, 9% for RIF, 6.1% for SM and 3% for ETB.[12] Moreover, total resistance rates in the subjects with lung tuberculosis were announced to be 20.7% for INH, 4.9% for RIF, 4.6% for SM and 2.7% for ETB.[12] In our study, however, total resistance rates in lung tuberculosis patients were observed to be 25% (16/64), 5% (3/64), 19% (12/64) and 5% (3/64) for INH, RIF, SM and ETB, respectively.

In various studies performed in Turkey in different years, resistance rates were reported to be 4.21-33.0% for INH, 1.5-39.5% for SM, 0-21.4% for ETB and 0-32.1% for RIF. The resistance rates determined in our study were 24%, 20%, 6% and 4% for INH, SM, ETB and RIF, respectively, and our rates were found to be in line with those determined in the studies reported.[7,11,12,14,16,20-39]

WHO emphasizes that the rates of primary MDR should be less than 1% in the countries where nationwide preventive schedules have been implemented effectively. However, in different studies performed in Turkey MDR was reported to be 1-5% between→

1999 and 2002 and to be 1.1-18.5% between 2003 and 2006 [6,7,11,12]. Upon evaluating the findings of studies performed on 55779 untreated patients in 75 in 75 countries/locations between 1999 and 2002, MDR was reported to be 1.1%, and primary resistant rates to one or more drugs were detected to be 10.2%. In the same study, the highest resistant rate to an antituberculosis drug was found out to be in Kazakhstan[6]. Secondary resistant rates during the same period were studied on treated 8400 patients in 66 countries/locations, and average resistant rate to one more drugs was reported to be 14.8% (the highest rate, 82.1% in Kazakhstan). Average secondary resistant rates were found to be 14.4% to INH, 11.4% TO SM, 8.7% to RIF and 3.5% to ETB[6], secondary resistant rates determined in our study were found to be 14% in INH, 10% in SM, 4% in RIF and ETB, and it was found out that these rates were in line with those found in other studies, performed in different countries. WHO reported the rate of MDR in secondary cases to be 7%[6]. In various studies performed in different countries between 1999 and 2002, MDR was reported to range from 1 to 13.7%[40]. MDR-TB, however, was reported to alter between 3.8% and 14.7% in various studies performed in Turkey[21-29]. MDR rates of 8% determined in patients displaying resistance in our study and of 3% determined in all tuberculosis patients were observed to be consistent with those detected in Turkey and reported by WHO. It was also determined that primary MDR of 0% and secondary MDR of 3% found in our study were consistent with the rates reported by WHO and also determined in other studies performed in Turkey. In the report in 2008, WHO explained that the countries where the highest rates of MDR-TB were found were Azerbaijan (22.3%), Moldovia (19.4%), Ukreine (16%), Russia (15%) and Uzbekistan (14.8%)[41,40].

The reason why various researchers reported different resistant rates, as with seen in those studies, was considered to stem from the fact that no standardization could be provided, resistant patterns could alter over the years, and method used in drug sensitivity tests were different.

CONCLUSIONS

Findings obtained in our study and from other studies performed in Turkey suggest that resistance rates are developing at alarming levels. Among the resistance rates to tuberculosis drugs reported from different regions in Turkey, there exist considerable differences concerning economic status, climate factors and level of development observed among the regions in Turkey. In terms of resistance rates, significant alterations are witnessed in different studies performed in different years in the same provinces. In order to determine reliable results and accurate resistance rates, methods used by different laboratories and drug concentrations should be standardized. The uncontrolled existence of many chronic and resistant tuberculosis untreated subjects in the society suggests that primary resistance challenge to tuberculosis drugs may lead to more serious problems in the future.

CORRESPONDING AUTHOR: Muhammet Güzel Kurtoğlu MD. Konya Education and Research Hospital, Meram Yeni Yol, KONYA kurtoglumg@hotmail.com

DELIVERING DATE: 18 / 10 / 2009 • **ACCEPTED DATE:** 23 / 03 / 2010

REFERENCES

1 Krugman S. Who news 200 million may die prematurely in the 1990. Bult Int Tuber Lung Dis 1990; 65: 101-102.

2 Brennan PJ. Structure of mycobacteria. Rev Infect Dis 1989; 12: 419- 421.

3 Çalışır HC. Tüberkülozda Direnç Sorunu ve Dirençli Tüberküloz Tedavisi. İnfek Hast Serisi 2001; 4: 47-56.

4 Özçelik U. Clinical findings and diagnosis in childhood tuberculosis. Türkiye Klinikleri J Pediatr Sci 2006; 2: 26-31.

5 Haas DW, Des Prez RM. Mycobacterium tuberculosis, Mandell GL. (eds.) Principles and Practice of Infectious Diseases. Churchill Livingstone, New York 1995: 2213-43

6 Durmaz R. Mycobacterium tuberculosis'de direnç sorunu. ANKEM 2005; 19: 107-110.

7 Özekinci T, Özbek E, Gedik M, Temiz H, Atmaca S. Drug resistance of Mycobacterium tuberculosis strains isolated between 2001-2003. J Turk Microbiol Soc 2006; 36: 31-34.

8 Uzunköy A, Nazlıgül Y. Peritoneal Tuberculosis. Turkiye Klinikleri J Med Sci 2006; 26: 404-408.

9 Çetin A, Göksedef PÇ, Görgen H, Tayhan M, Şencan D. Peritoneal tuberculosis mimicking advanced stage ovarian carcinoma: presentation of two cases. Turkiye Klinikleri J Gynecol Obst 2005; 15: 36-39

10 Anğ Ö, Erturan Z, Uzun M. Dünyada ve Türkiye'de tüberküloz. Turk J Infect 1997; 11: 1-5.

11 Durmaz R, Ozerol IH, Durmaz B, et al. Primary drug resistance and molecular epidemiology of Mycobacterium tuberculosis isolates from patients in a population with high tuberculosis incidence in Turkey. Microb Drug Resist 2003; 9: 361-366.

12 Doğan ÖT, Özşahin SF, Kaya S, Bakıcı MZ, Yaldız AI. Anti tuberculosis drug resistance in consecutive 385 patients followed since 1999 in Cumhuriyet University Hospital. C. U. Tıp Fak Derg 2004; 26: 81-84.

13 Loddenkemper R, Sagebiel A, Brendel A. Strategies against multidrug-resistant tuberculosis. Eur Respir J 2002; 20: 66-77.

14 Kömürcüoğlu B, Şenol G, Balcı G, Yalnız E, Özden E. Drug Resistance in Pulmonary Tuberculosis; in New and Previously Treated Cases. The Arch of Lung 2007; 8: 117-121.

15 Çalışır H, Ulukavak T, Türker G, Özkara Ş, Öğretensoy M. Tüberkülozda tedavi başarısızlığına neden olan etkenler. Sol Hast 1997; 8: 225-233.

16 Tahaoğlu K, Kızkın Ö, Karagöz T. High initial and acquired drug resistance in pulmonary tuberculosis in Turkey. Tuberc Lung Dis 1994; 75: 324-328.

17 Kılıçaslan Z, Sarımurat N, Ortaköy G. Multidrug resistant tuberculosis in İstanbul. Tuberc Lung Dis 1994; 75: 15-17.

18 Kartaloğlu Z, Bozkanat E, Öztürkeri H, Okutan O, İlvan A. BACTEC

yöntemi kullanılarak primer antitüberküloz ilaç direnci saptanan 365 tüberküloz olgusu. Sol Derg 2002; 4: 443-448.
19 Yolsal N, Malat G, Dişçi R, Örkün M, Kılıçaslan Z. Türkiye'de tüberküloz ilaçlarına direnç sorununun 1984-1989 ve 1990-1995 yılları için karşılaştırılması: Meta-analiz. KLIMIK 1998; 11: 6-9.
20 Talay F, Altın S, Karasu L, Kümbetli Ş. İstanbul Eyüp Verem Savaş Dispanserinde 1997-2000 yıllarında belirlenen ilaç direnç oranları. Van Med J 2003; 10: 10-15.
21 Ozturk CE, Balbay OA, Kaya D, et al. The resistance to major antituberculous drugs of Mycobacterium tuberculosis strains isolated from the respiratory system specimens of tuberculosis patients in Duzce, Turkey. Jpn J Infect Dis 2005; 58: 47-49.
22 Şenol G, Komurcuoglu B, Komurcuoglu A. Drug resistance of Mycobacterium tuberculosis in western Turkey: a retrospective study from 1100-bed teaching hospital. J Infect 2005; 50: 306-311.
23 Surucuoglu S, Ozkutuk N, Celik P, et al. Drug-resistant pulmonary tuberculosis in western Turkey: prevalence, clinical characteristics and treatment outcome. Ann Saudi Med 2005; 25: 313-318.
24 Saygan MB, Ocak F, Cesur S, et al. Susceptibilities of Mycobacterium tuberculosis strains collected from regional tuberculosis laboratories to major antituberculous drug. Mikrobiol Bul 2007; 41: 403-409.
25. Saral OB, Sucu N, Boz GA, Erdem M, Köksal I. 442 Mycobacterium tuberculosis suşunda BACTEC yöntemi ile kombine ilaç direncinin araştırılması. Tur Toraks Der 2007; 8: 174-178.
26 Şenol G, Coşkun M, Biçmen C, Erer OF. Aktif akciğer tüberkülozlu hastalardan izole edilen Mycobacterium tuberculosis kökenlerinin ilaç direnç oranlarının değerlendirilmesi. Klimik Dergisi 2006; 9: 71-74.
27 Aslan G, Delialioğlu N, Emekdaş G, et al. Mycobacterium tuberculosis suşlarının izoniazid, rifampisin, streptomisin ve etambutol duyarlılıklarının BACTEC yöntemi ile belirlenmesi. ANKEM 2005; 19: 43-47.
28 Durmaz R, Zozio T, Gunal S, et al. Population-based molecular epidemiological study of tuberculosis in Malatya, Turkey. J Clin Microbiol 2007; 45: 4027-4035.
29 Gönlügür U, Bakici MZ, Gönlügür TE, Hasbek M, Resistance rates to antituberculous drugs in Sivas province. Mikrobiyol Bul 2007; 41: 459-463.
30 Uçar E, Kılıç A, Ceyhan İ, et al. Ülkemizin yedi farklı bölgesinden 2003-2006 yılları arasında izole edilen Mycobacterium tuberculosis suşlarının majör antitüberküloz ilaçlara direnç oranları. Mikrobiyol Bul 2010; 44: 11-19.
31 Sarıbaş S, Bağdatlı Y, Yıldız N, Yiğit Ö. Mycobacterium tuberculosis' de direnç sorunu 166 suşun dört major ilaca dirençlerinin araştırılması. Turk J Infect 2004; 18: 447-449.
32 Tansel Ö, Yüksel P, Kuloğlu F, Akata F. Mycobacterium tuberculosis'in antitüberküloz ilaçlara direnci: Trakya Üniversitesi hastanesinin iki yıllık Sonuçlarının değerlendirilmesi. Tur J Infect 2003; 17: 23-26.
33 Orhan G, Zer Y, Balcı İ, Bayram A, Korkmaz G. Mikobakteriyoloji laboratuarında incelenen örneklerin retrospektif olarak değerlendirilmesi. J Turk Microbiol Soc 2002; 32: 225-229.
34 Güneri S, Ünsal İ, Öztop A, et al. Mycobacterium tuberculosis suşlarının antituberküloz ilaçlara direnç oranları: Ege bölgesine ait iki yıllık verilerin değerlendirilmesi. Mikrobiyol Bult 2004; 38: 203-209.
35 Baylan O, Albay A, Özgül K, Tekbaş ÖF, Deniz Ö. 2002-2003 yıllarında izole edilen Mycobacterium tuberculosis kompleks suşlarında primer ilaç direnç oranlarının saptanması ve1998-2001 verileriyle karşılaştırılması. Mikrobiyol Bult 2005; 39: 153-160.
36 Şenol G, Kömürcüoğlu B, Kömürcüoğlu A. Mycobacterium tuberculosis kökenlerin antitüberküloz ilaçlara direnç durumu. Turk J Infect 2004; 18: 441-444.
37 Yaylı G, Hamdi S, Canan A. Isparta yöresinde izole edilen Mycobacterium tuberculosis suşlarının antituberküloz ilaçlara duyarlılıkları. J Turk Microbiol Soc 2003; 33: 24-30.
38 Otkun M, Akata F, Karabay O, et al. Trakya Üniversitesi Hastanesine 1996 yılı içinde başvuran tüberkülozlu olgularda antitüberküloz ilaçlara direnç sorunu, Turk J Infect 1997; 11: 191-196.
39 Saniç A, Günaydın M, Çoban AY, Tokaç MS, Çetin M. A comparison of the E-test and proportion methods for susceptibility testing of Mycobacterium tuberculosis, J Chemother 2000; 12: 491-494.
40 Falzon D, Kudjawu Y, Desenclos JC, et al. Stopping TB in Europe: some progress but still not there. Euro Surveill 2008; 13: 8073.
41 World Health Organization. Tuberculosis, MDR-TB & XDR-TB. 2008 Report. Anti-tuberculosis drug resistance in te world. (www.who.int/entity/tb/features_archive/drs_factsheet.pdf)