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Characterization of Resistance Mutations Against First and Second Line Antituberculosis Drugs of Multi-Drug Resistant *Mycobacterium tuberculosis* Strains Isolated from Clinical Samples by in-House PCR Method and Comparison of Resistance Profiles

Klinik Örneklerden İzole Edilen Çok İlaca Dirençli *Mycobacterium tuberculosis* Suşlarının Birinci ve İkinci Kuşak Antitüberküloz İlaçlara Karşı Direnç Mutasyonlarının In-house PCR Yöntemiyle Karakterizasyonu ve Direnç Profillerinin Karşılaştırılması

Sümeyye ÇAPUK(İD), Gülnur TARHAN(İD)

Department of Medical Microbiology, Adıyaman University Faculty of Medicine, Adıyaman, Türkiye

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ABSTRACT

Introduction: *Multi-drug resistant tuberculosis (MDR-TB) is still an important public health problem. Rapid agent diagnosis and resistance status determination are critical in establishing the correct treatment protocol. This study was conducted to determine resistance mutations against first- and second-line antituberculous drugs in the MDR-MTB strain isolated from respiratory tract specimens.*

Materials and Methods: *After subculturing the isolated Mycobacterium tuberculosis strains on the Löwenstein-Jensen medium, DNA isolation was carried out using the boiling method. DNA isolates were kept at -40° C until the day of analysis. Primer sequences specific to rpoB, InhA, katG, gyrA, eis, and rrs regions were used to determine isoniazid, rifampicin, quinolone, and aminoglycoside resistance.*

Results: *The positivity rate of rpoB, InhA, katG, gyrA, eis, and rrs in 33 MDR-TB isolates was 27 (81.8%), 31 (93.9%), 25 (75.7%), 25 (75.7%), 20 (60.6%) and 14 (42.4%), respectively. Resistance mutations were not detected in susceptible isolates.*

Conclusion: *According to the data obtained from the study, it was found that fluoroquinolone resistance mutations were higher in isolates defined as MDR-TB by conventional and molecular methods, and the in-house PCR method was a useful method for rapid resistance detection.*

Key Words: *MDR-TB; Mutation; Mycobacterium tuberculosis; PCR*

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ÖZ

Klinik Örneklerden İzole Edilen Çok İlaca Dirençli Mycobacterium tuberculosis Suşlarının Birinci ve İkinci Kuşak Antitüberküloz İlaçlara Karşı Direnç Mutasyonlarının in-House PCR Yöntemiyle Karakterizasyonu ve Direnç Profillerinin Karşılaştırılması

Sümeyye ÇAPUK, Gülnur TARHAN

Adıyaman Üniversitesi Tıp Fakültesi, Tıbbi Mikrobiyoloji Anabilim Dalı, Adıyaman, Türkiye

Giriş: *Çok ilaca dirençli tüberküloz (ÇİD-TB) hala önemli bir halk sağlığı sorunudur. Etkenin hızlı teşhisi ve direnç durumunun belirlenmesi, doğru tedavi protokolünün oluşturulmasında kritik öneme sahiptir. Bu çalışma, solunum yolu örneklerinden izole edilen ÇİD-TB suşunda birinci ve ikinci basamak antitüberküloz ilaçlara karşı direnç ile ilişkili mutasyonları belirlemek amacıyla yapılmıştır.*

Materyal ve Metod: *İzole edilen mikobakteri suşlarının Löwenstein-Jensen besiyerinde alt kültürü yapıldıktan sonra, kaynatma yöntemine göre DNA izolasyonu yapıldı. DNA izolatları çalışma gününe kadar -40° C'de muhafaza edildi. İzoniazid, rifampisin, kinolon ve aminoglikozid direncini belirlemek için rpoB, InhA, katG, gyrA, eis ve rrs bölgelerine özgül primer dizileri kullanıldı.*

Bulgular: *Otuz üç ÇİD-TB izolatında rpoB, InhA, katG, gyrA, eis ve rrs pozitiflik oranı sırası ile 27 (%81.8), 31 (%93.9), 25 (%75.7), 25 (%75.7), 20 (%60.6) ve 14 (%42.4) olarak bulundu. Duyarlı izolatlarda direnç ile ilişkili mutasyonlar tespit edilmedi.*

Sonuç: *Bu çalışmadan elde edilen verilere göre, konvansiyonel ve moleküler yöntemlerle MDR-TB olarak tanımlanan izolatlarda florokinolon direnci ile ilgili mutasyonların daha yüksek olduğu ve in-house PZR yönteminin hızlı direnç tespiti için faydalı bir yöntem olduğu saptanmıştır.*

Anahtar Kelimeler: *ÇİD-TB; Mutasyon; Mycobacterium tuberculosis; PZR*

INTRODUCTION

Tuberculosis (TB) is a highly significant global health concern, as it remains one of the deadliest diseases caused by a single infectious agent. Each year, millions of individuals worldwide are still being infected with TB[1]. According to the 2021 Global Tuberculosis Report by the World Health Organization (WHO), it was documented that around 5.8 million individuals were affected by tuberculosis (TB) in 2020[2]. Although effective control and treatment programs continue all over the world, it is not possible to control the disease at the desired level due to reasons such as rapid population growth, wars, continuous migration, increasing rates of immunodeficiency, and poverty[3]. Antituberculosis drug resistance, which emerged in the early 1990s, has become a significant global health challenge. It poses a substantial threat to the success of tuberculosis control programs, particularly those targeting multi-drug resistant tuberculosis (MDR-TB), and hinders efforts to effectively manage the disease[4]. According to the 2021 WHO report, a total of 150.359 individuals with multidrug-resistant or rifampicin-resistant tuberculosis (MDR/RR-TB) were enrolled in treatment worldwide in the year 2020[2]. As per the 2020 report released by the Tuberculosis Association of Türkiye, the prevalence of rifampicin-resistant (RR)/MDR-TB in Türkiye was estimated to be 3.5% among new cases and 12% among previously treated cases[5]. In patients with MDR-TB, the addition of second-line drugs to first-generation drugs leads to prolongation of treatment[6]. The fact that second-line anti-tuberculosis drugs, when added to the treatment regimen, have more pronounced toxic effects and lower efficacy, not only results in the occurrence of numerous side effects in patients but also has a negative impact on patient compliance[7]. In addition, the cost of second-line drugs is another reason for patients to be negatively affected[8]. Drug resistance in *Mycobacterium tuberculosis complex* (MTBC) develops through many mechanisms, albeit with a varying frequency for each antibiotic. These mechanisms include enzyme inactivation, decrease in permeability, change in antibiotic target, increased efflux mechanisms, and decrease in the activity of enzymes that activate prodrugs[9,10]. Determining resistance quickly and accurately is extremely important in preventing antituberculosis drug resistance[8].

Therefore, in studies conducted, nucleic acid diagnostic tests that detect resistance rapidly and accurately should be developed to determine the appropriate antituberculosis treatment protocol for the patient[11].

This study was carried out to determine the rpoB, inhA, katG, gyrA, eis, and rrs gene regions of major first and second-generation anti-TB drugs in MDR-TB isolates by in-house PCR method.

MATERIALS and METHODS

Clinical isolates: In this study, 10 susceptible MTB strains isolated from sputum samples in Adıyaman Üniversitesi Training and Research Hospital and 33 MDR-MTB isolates voluntarily obtained from Atatürk Chest Training and Research Hospital, Ankara between 2017-2018 were evaluated by using in-house PCR.

Drug susceptibility: First- and second-generation drug susceptibility tests of clinical isolates were performed in the Lowenstein-Jensen medium by indirect proportion method. For these tests, the following concentrations were used: 0.2 µg/mL for isoniazid, 40 µg/mL for rifampicin, 4 µg/mL for streptomycin, 2 µg/mL for ethambutol, 0.2 µg/mL for second-generation drugs such as ofloxacin, 0.5 µg/mL for moxifloxacin, 40 µg/mL for amikacin, and 40 µg/mL for kanamycin. H37Rv ATCC 27294 reference strain was used as a positive control for each drug susceptibility test. All test tubes were incubated for 6 to 8 weeks in an incubator set at 37° C. During the incubation period, growth control was performed twice a week. The growing bacterial colonies were collected using a loop and suspended in an Eppendorf tube containing TE buffer. The suspension was then stored at -20° C until DNA isolation[12,13].

DNA isolation: MTB DNA was isolated using the boiling method[14]. For this purpose, a loopful of bacterial colonies grown from the LJ medium were suspended in an Eppendorf tube containing 750 µL TE buffer. After mixing thoroughly with vortex, the upper liquid was discarded by centrifugation at 10.000xg for 10 minutes. This process was repeated three times to wash the bacteria. The bacteria solution was suspended by adding 250 µL TE buffer for the last time and kept in a boiling water bath for 20 minutes, allowing the bacteria to be broken down and DNA to be released. The tubes were centrifuged at 10.000xg for 10 minutes, allowing bacterial residues to collapse. The upper liquid containing the template DNA was removed and placed in a sterile microcentrifuge tube. The extracted DNA was stored at -40° C for PCR amplification.

In-house PCR Test: The in-house PCR test was used to investigate the presence of rpoB mutations associated with rifampicin resistance, as well as katG and inhA mutations related to isoniazid resistance. Additionally, gyrA mutations were examined to determine drug resistance in the fluoroquinolone group. The eis and rrs gene regions were targeted to detect resistance in the aminoglycoside group. The forward and reverse primer sequences used in the identification of each resistance gene are given in Table 1.

The tests were conducted in a volume of 50 µL. The reaction mixture consisted of 28.8 µL distilled water, 5 µL 10x PCR buffer, 4 µL (2 mM) MgCl2, 1 µL dNTP mix, 0.5 µL of each primer (R and G), 0.2 µL Taq DNA polymerase, and 10 µL template DNA. All tubes were incubated for 35 cycles at 94° C for 1 min, at 60° C (58° C for rpoB primer only) for 1 min, and at 72° C for 1 min, respectively. After this step, the reaction tubes were incubated at 72° C for 10 minutes. Following the incubation period, the amplification process was terminated. PCR products were run in 2% agarose gel electrophoresis in the presence

Table 1. Primer sequences used in the study

rpoB	F:5'ACCGACGACATCGACCACTT-3' , R:5'-GGCGGTCAGGTACACGATCT-3'
katG	F:5'-GAGCCCGATGAGGTCTATTG-3' R:5'-GTCTCGGTGGATCAGCTTGT-3'
inhA	F:5'-CGCAGCCAGGGCCTCGCTG-3, R:CTCCGGTAACCAGGACTGA-3'
gyrA	F: 5' -TCGACTATGCGATGAGCGTG-3', R: 5'-GGTAGCACCGTCGGCTCTTG-3'
rrs	F:5'-GAGTTGGTGCGGCGTAAGAGC-3', R: 5' -GGGGCGTTTTCGTGGTGCTCC-3'
eis	F: 5'-GCGTAACGTCACGGCGAAATTC-3' ,R: 5'-GTCAGCTCATGCAAGGTG-3'

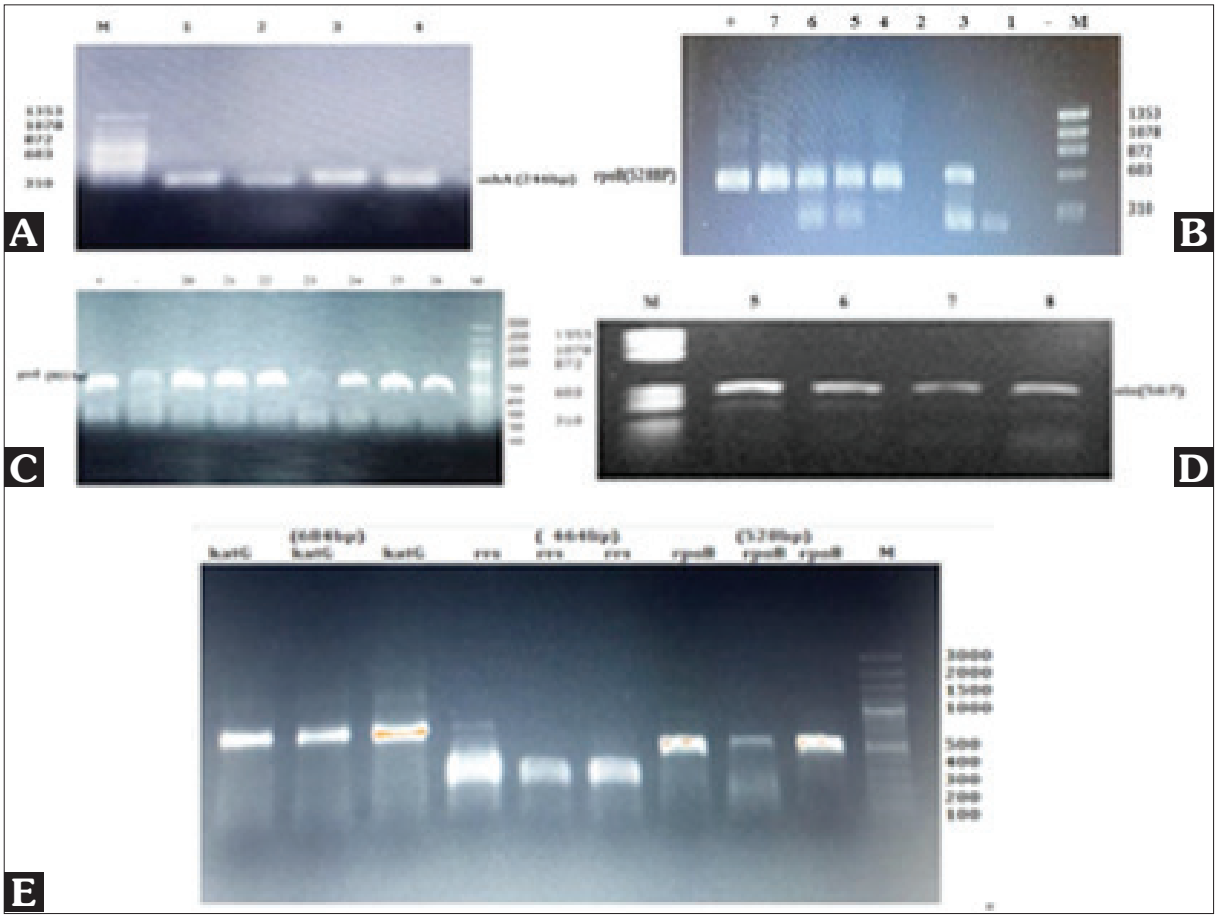

Figure 1. Electrophoresis image of resistance gene regions by PCR test in MDR-MTB isolates A) InhA B) rpoB: C) gyrA D) eis E) katG, rrs, rpoB.

M: Marker GeneRuler100 bp Plus DNA marker and X174 DNA/HinfI Marker, +: Positive control, -: Negative control.

of ethidium bromide at 90 V for one hour. The DNA bands obtained were compared with molecular weight markers (100 bp DNA marker, GeneRuler 100 bp Plus DNA marker, and X174 DNA/HinfI Marker) in the UV transilluminator gel documentation system. Positive control and negative control were used in each study. Bands with a size of catG 684 bp, rpoB 528 bp, inhA 246 bp, gyrA 415 bp rrs 464 bp, and eis 567 bp were considered positive (Figure 1).

RESULTS

Resistance profiles of 43 MTB strains against first- and second-line anti-TB drugs were studied by indirect proportion method in LJ medium. All MDR-TB strains included in the study were found to be resistant to first-line drugs INH and RIF.

The resistance rate of isolates to ofloxacin in the fluoroquinolone group of second-line drugs was found as 3/33 (9.09%), while all isolates were susceptible to moxifloxacin. The resistance rate against amikacin and kanamycin, which are injectable drugs in the aminoglycoside group, was determined as 1/33 (3.03%).

The result of the conventional DST and in-house PCR of the 33 MDR-TB isolates are given in Table 2.

According to these results, 27 (81.8%) of the 33 isolates examined had the rpoB gene, 31 (93.9%) isolates had the inhA gene, and 25 (75.7%) isolates were positive for the katG gene. InhA and katG genes of six isolates with negative rpoB gene were positive.

Table 2. Comparison of indirect proportion and PCR test results in MDR-MTB isolates

Pharmaceutical Groups		Test results: in-House PCR	Test results: Conventional IDT
Rifampicin (rpoB)		27/33 (81.8%)	33/33 (100%)
Isoniazid	(ınhA)	31/33 (93.9%)	33/33 (100%)
	(katG)	25/33 (75.7%)	
FQ (gyrA) (Ofloxacin Moxifloxacin)		25/33 (75.7%)	3/33 (9.09%)
Aminoglycosides	(eis)	20/33 (60.6%)	1/33 (3.03%)
Amikacin Kanamycin	(rrs)	14/33 (42.4%)	

The rpoB gene was found to be positive in three isolates with negative inhA results and eight isolates with negative katG results. In our study, none of the isolates detected susceptible to rpoB, inhA, and katG were found to be resistant in combination with one or both of rpoB inhA and/or katG. In one isolate that tested positive for inhA and katG, the rpoB gene was found to be negative. Of 18 (54.5%) isolates; rpoB, katG and INH resistance was positive.

The gyrA gene region, which indicates the presence of mutations associated with fluoroquinolone group drugs, showed a positivity rate of 75.7% (25/33). In the aminoglycoside group, the eis gene region representing mutational resistance regions was found to be positive in 20 (60.6%) of 33 isolates and 14 (42.4%) of the rrs gene region. In our study, all gene regions examined were found to be susceptible in 10 *Mycobacterium tuberculosis* (MTB) isolates that were susceptible to the first and second-line drugs used for control purposes. Positivity was detected in the rpoB gene region of 21 of the isolates and one of the katG or inhA regions. It was determined that these isolates were resistant to one of the gyrA gene region and eis and/or rrs gene regions. GyrA mutational gene region was not detected by PCR method in 1 ofloxacin-resistant isolate according to the indirect proportion method.

Table 3 presents the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and agreement values between the two tests of the in-house PCR test for each drug. These parameters were calculated by evaluating a total of 33 MDR-MTB isolates and 10 MTB isolates used as controls.

Table 3. The sensitivity, specificity, PPD, NPD and test compliance values of the PCR method

Tested Gene Region	True (+)	True (-)	False (+)	False (-)	Sensitivity	Specificity	PPD	NPD	Test Compliance
rpoB	27	10	-	6	81	100	100	62	86
inhA	31	10	-	2	93	100	100	83	95
katG	25	10	-	8	75	100	100	55	81
gyrA	1	17	23	2	33	42	4	89	41
eis	1	23	19	-	100	54	5	100	55
rrs	1	29	13	-	100	69	7	100	69

Sensitivity= True positive value/True positive value + False negative value.
Specificity= True negative value/True negative value + False positive value.
Positive predictive value= True positive value/True positive value + False positive value.
Negative predictive value= True negative value/True negative value + False negative value.
Agreement of tests= Total of positives in both methods + Total of negatives in both methods/Total number of samples.

According to these results, the sensitivity and specificity rate for detecting the rpoB gene for rifampicin resistance was 81% and 100%, respectively. Sensitivity rates for inhA and katG gene detection for isoniazid resistance were 93% and 75%. The agreement rate between both tests was 86%, 95%, and 81% for rpoB, inhA, and katG, respectively. The sensitivity for gyrA, the fluoroquinolone group resistance detection genes among the second-line drugs, was 33% and the test agreement was 41%. Sensitivity rates for eis and rrs genes in the aminoglycoside group were 100% and 100%, respectively. The test compliance rates in this group were 55% and 69%.

DISCUSSION

Multidrug-resistant tuberculosis is becoming an increasingly significant public health issue both globally and within our country. Although it is expensive, complex, and difficult to intervene, it is a curable disease. In the treatment of MDR-TB, fluoroquinolone and aminoglycoside group drugs are administered in combination with first-line drugs. However, it has been reported that resistance has also developed against these drugs since the emergence of MDR-TB[15-18].

According to the Global Tuberculosis Report published by WHO in 2021, 157.903 of the cases were detected as pre-XDR-TB or XDR-TB cases[5]. To prevent new MDR-TB cases, early and accurate diagnosis of these cases and performing first- and second-line drug sensitivity tests in MDR-TB treatment is critical. Today, there are culture-based methods and molecular tests used for this purpose. However, this method causes serious delays due to the need for the growth of bacteria in solid or liquid media used in traditional drug sensitivity methods (indirect proportion, absolute concentration method) and the slow growth of mycobacteria. Many studies have reported that the application of in-vitro tests, especially for second-line drugs, is laborious, difficult to interpret, and performed in a limited number of centers. For these reasons, rapid molecular detection methods have become an important need in the definition of resistance[19]. Given that commercial molecular tests such as Xpert MTB/RIF, GenoType MTBDR/MTBDRplus, and sequence analysis used for this purpose are costly and require infrastructure. Researches are ongoing to develop rapid, affordable, and practical tests for the identification of drug resistance[20,21].

Our study aimed to identify the presence of resistance genes against fluoroquinolone and aminoglycoside drugs in isolates with MDB-MTB detected by conventional methods by in-house PCR method and to evaluate their effectiveness.

In our study, out of the 33 isolates diagnosed with MDR-TB using conventional methods, 27 (81.8%) showed positive results for the rpoB gene region, 31 (93.9%) for the inhA gene region, and 25 (75.7%) for the katG gene region. Interestingly, while katG mutations are commonly reported as the primary cause of INH resistance in previous studies, our study observed a higher frequency of InhA mutations as the cause of INH resistance[21,22].

In their 2018 study conducted in Morocco, Oudghiri et al. evaluated drug resistance in 703 Mycobacterium tuberculosis (MTB) strains. As a result of the drug sensitivity test performed against first-generation drugs, 68.6% (482/703) of the 703 strains were found to be RIF- and/or INH-resistant. In the study, the isolates used were categorized as follows: 28.2% (198/703) were resistant to RIF alone, 27.6% (194/703) were resistant to INH alone, and 12.8% (90/703) were identified as MDR-TB. Susceptibility tests were conducted on the strains identified as MDR-TB against second-generation drugs. Among these strains, 20 out of 90 (22.2%) were found to be resistant to fluoroquinolone group drugs. Additionally, 2 out of 90 (2.22%) were resistant to kanamycin, and 3 out of 90 (3.33%) were resistant to amikacin[23].

In the study conducted by Gupta et al. in Beijing in 2020, a total of 76 Mycobacterium tuberculosis (MTB) isolates were evaluated using Multiplex-PCR. The findings revealed that 47 (61.8%) isolates were resistant to RIF, 50 (65.7%) were resistant to INH, and 45 out of 76 (59.2%) isolates were identified as MDR-TB[24].

In studies where fluoroquinolone resistance was investigated globally, it was found that 60% to 90% of mutations were in codon 88 and codon 90[25]. The mechanism of resistance to fluoroquinolones is point mutations in the gyrA and gyrB genes, and DNA encodes two subunits of gyrase. While most mutations that provide resistance to fluoroquinolones occur in a short segment in the gyrA gene called the Quinolone Resistance Determining Region (QRDR), studies have shown that gyrB mutations occur less frequently in fluoroquinolone resistance[24-26]. In our study, the gyrA gene region was found to be 75.7% (25/33) in the evaluation performed in 33 MDR-TB isolates by PCR test. Fluoroquinolone group drugs are the mainstay in the treatment of MDR-TB and XDR-TB patients; however, fluoroquinolone resistance tends to increase gradually and prevents the success of the National Tuberculosis Control Programs[27].

Our fluoroquinolone resistance rate was found to be similar to Shanghai (76.0%). It was lower than Russia (83.0%), India (81.0%) and Thailand (92.3%), and higher than Morocco (30.0%) and New York (67.0%)[28-33].

In the study conducted by Li et al., a total of 257 isolates were evaluated using spoligotyping and MIRU-VNTR methods. Among these isolates, 170 (66.1%) were found to have INH resistance, and 152 (59.1%) were identified as RIF resistant. The number of isolates resistant to fluoroquinolone group drugs was reported as 97 (37.7%). At the same time, the resistance of 257 isolates to ofloxacin, levofloxacin, kanamycin, and amikacin was determined as 84 (32.7%), 56 (21.7%), 52 (20.2%), and 18 (7.0%), respectively[34].

In the study conducted by Ramarkarishma et al. in 2020, 13 MDR-TB isolates were evaluated and mutation in the eis region of these isolates was found to be 7/13 (53.9%) in high frequency. The rrs mutation in this study was reported as 2/13 (15.4%)[35]. In our study, it was detected with a higher rate of 60.6% in the eis gene region.

Kateete et al. detected gyrA and gyrB mutations in 72.2% of 38 MDR-TB isolates in their study in 2019 in Somalia and Uganda. Additionally, drug resistance mutations in rrs have been reported to occur in 64.3% of second-line anti-TB injectable drug-resistant MDR isolates[36]. In our study, it was found that out of the isolates used, 60.6% (20/33) exhibited resistance in the eis gene region, while 42.4% (14/33) showed resistance in the rrs gene region. These study results are in agreement with the results of our study.

Kanamycin, amikacin, and capreomycin, which are among the aminoglycoside group drugs, are considered key drugs in MDR-TB treatment. The increasing rates of resistance to these drugs and possible cross-resistance among them cause important problems in MDR-TB treatment. In studies conducted in recent years, it has been reported that there is increasing resistance to aminoglycoside drugs in MDR-TB cases[37,38]. Studies have reported that mutations in the Eis promoter region are highly related to KAN resistance. It has been shown that mutations in the rrs gene encoding the 16S rRNA bacterial subunit confer high levels of resistance to KAN, cross-resistance to AMK, and sometimes CAP[39]. Of the isolates evaluated in our study, 21 (63.6%) were positive for rpoB, and it was found that these isolates were resistant to one of the inhA or katG regions and additionally to gyrA. Out of the isolates used in the study, 54.5% (18/33) were found to be positive for all three resistance genes (rpoB, katG, and inhA). Additionally, 21 isolates exhibited resistance in both the rpoB and gyrA genes, while one isolate showed mutation positivity in the katG-inhA region. Furthermore, one isolate displayed resistance in the eis-rrs gene region, indicating the presence of XDR-TB.

It is thought that these differences between conventional methods and in-house PCR may be the reasons that negatively affect the determination of the resistance, which is too low to be detected by conventional (phenotypic) methods, molecular methods, and the performance of sensitivity tests in the LJ medium (for example, high protein content binding antibiotics).

When conventional indirect drug susceptibility testing (IDT) in the Löwenstein-Jensen medium was considered the gold standard for first- and second-line drugs, the sensitivity of the PCR test in detecting the rpoB gene for rifampicin (a first-generation drug) was 81%. The sensitivity for detecting the inhA and katG genes for isoniazid was 93% and 75%, respectively. In terms of the PCR test's sensitivity in determining the resistance genes gyrA, eis, and rrs for second-generation drugs such as fluoroquinolones and aminoglycosides, the rates were 33%, 100%, and 100% respectively. The agreement rate between the two tests was between 81-95% for first-generation drugs and 41-69% for second-generation drugs.

Based on the results obtained in our study, it is not appropriate to rely solely on the in-house PCR test for detecting resistance. Instead, it can be utilized as a complementary or pre-screening test alongside conventional drug susceptibility tests in the identification of resistance. However, it is crucial to emphasize the need for proper standardization of the test for accurate and reliable results.

Due to the high fluoroquinolone resistance and aminoglycoside resistance of 33 MDR-TB isolates evaluated, more stringent measures should be taken against MDR-TB and antibiotics should be used more regularly. Insufficient detection of resistance rates against second-generation drugs may cause the actual resistance rates to remain hidden. Clinicians interested in the treatment of pulmonary tuberculosis should consider the quinolone resistance in our country when creating a treatment plan.

CONCLUSION

In conclusion, it is crucial to establish a standardized method for determining resistance to second-generation drugs in every patient and region across our country. This would facilitate the generation of extensive data on drug resistance. Since our study involved a limited number of MDR-TB cases, larger studies should be conducted, and the findings should be supported by DNA sequence analysis. Furthermore, we believe that identifying resistance mutations in both the GyrA and gyrB gene regions will contribute to a more precise determination of fluoroquinolone resistance.

ETHICS COMMITTEE APPROVAL

Ethics committee approval is not needed for this study since the data is dated before 2020.

CONFLICT of INTEREST

None of the authors had conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: GT

Analysis/Interpretation: SÇ

Data Collection or Processing: GT, SÇ

Writing: GT, SÇ

Review and Correction: GT

Final Approval: GT

REFERENCES

1. *Baylan O. Extensively drug resistant and extremely drug resistant tuberculosis forms after multi-drug resistant tuberculosis: New faces of the old disease. Mikrobiyol Bul 2011;45:181-95.*
2. *World Health Organization (WHO). Global tuberculosis report 2019. Available from: https://www.who.int/tb/publications/global_report/en/ (Accessed date: 25.10.2019).*
3. *Kisa O, Albay A, Baylan O, Balkan A, Doganci L. Drug resistance in Mycobacterium tuberculosis: A retrospective study from a 2000-bed teaching hospital in Ankara, Turkey. Int J Antimicrob Agents 2003;22:456-7. https://doi.org/10.1016/S0924-8579(03)00160-2*
4. *Dündar D, Sönmez TG. Mycobacterium tuberculosis kompleksi izolatlarının primer antitüberküloz ilaçlara direnç oranları. Klimik Derg 2009;22:52-4.*
5. *T.C. Sağlık Bakanlığı. Türkiye'de verem savaşı 2018 raporu. In: Kara F (ed). Ankara: Artı6 Medya Matbaacılık, 2019:65.*
6. *Aksu M, Şaşmaz T, Bayrak H. Akciğer tüberkülozlu olgulardan izole edilen Mycobacterium tuberculosis izolatlarında primer tüberküloz ilaç direncinin araştırılması. ESTÜDAM Halk Sağlığı Derg 2019;4:122-30. https://doi.org/10.35232/estudamhsd.524166*
7. *Bektöre B, Haznedaroğlu T, Baylan O, Özyurt M, Özkütük N, Şatana D, ve ark. Çok ilaca dirençli tüberküloz izolatlarında yaygın ilaç direncinin araştırılması. Mikrobiyol Bul 2013;47:59-70. https://doi.org/10.5578/mb.4229*
8. *Çilli A. Antitüberküloz ilaçlar ve etki mekanizmaları. 21. yüzyılda tüberküloz sempozyumu ve II. tüberküloz laboratuvar tanı yöntemleri kurs kitabı. Samsun: Ofset Basım, 2003;163-70.*

9. *Wade MM, Zhang Y. Mechanisms of drug resistance in Mycobacterium tuberculosis. Front Biosci 2004;9:975-94. https://doi.org/10.2741/1289*

10. *Caminero JA, Sotgiu G, Zumla A, Migliori GB. Best drug treatment for multidrugresistant and extensively drug-resistant tuberculosis. Lancet Infect Dis 2010;10:621-9. https://doi.org/10.1016/S1473-3099(10)70139-0*

11. *Durmaz R. Tüberkülozda hızlı moleküler tanı testleri. ANKEM Derg 2012;26:72-81. https://doi.org/10.5222/ankem.2012.176*

12. *Canetti G, Rist N, Grosset J. Measurement of sensitivity of the tuberculous bacillus to antibacillary drugs by the method of proportions. Methodology, resistance criteria, results and interpretation. Rev Tuberc Pneumol (Paris) 1963;27:217-72.*

13. *Şimşek H, Tarhan G, Cesur S. Çok ilaca dirençli Mycobacterium tuberculosis kompleks izolatlarında ikinci basamak antitüberküloz ilaç duyarlılıklarının E-test yöntemi ile değerlendirilmesi. Mikrobiyol Bul 2015;49:47-55. https://doi.org/10.5578/mb.8602*

14. *Ergin A. Kocagöz T, Us D. Evaluation of 120 mycobacterial strains isolated from clinical specimens to the species level by polymerase chain reaction-restriction enzyme analysis. Scand J Inject Dis 2000;32:657-62. https://doi.org/10.1080/003655400459586*

15. *Terzi HA, Aydemir Ö, Karakeçi E, Köroğlu M, Altındiş M. M. tuberculosis kompleks izolatlarının antitüberküloz ilaçlara direnç oranlarında yıllara göre değişim. Online Türk Sağlık Bilimleri Derg 2019;4:47-56. https://doi.org/10.26453/otjhs.441168*

16. *Dheda K, Gumbo T, Gandhi NR, Murray M, Theron G, Udwadia Z, et al. Global control of tuberculosis: From extensively drug-resistant to untreatable tuberculosis. The Lancet Respir Med 2014;2:321-38. https://doi.org/10.1016/S2213-2600(14)70031-1*

17. *Lin HC, Perng CL, Lai YW, Lin FG, Chiang CJ, Lin HA, et al. Molecular screening of multidrug-resistance tuberculosis by a designated public health laboratory in Taiwan. Eur J Clin Microbiol Infect Dis 2017;36:2431-9. https://doi.org/10.1007/s10096-017-3082-9*

18. *Becerra MC, Appleton SC, Franke MF, Chalco K, Arteaga F, Bayona J, et al. Tuberculosis burden in households of patients with multidrug-resistant and extensively drug-resistant tuberculosis: A retrospective cohort study. The Lancet 2011;377:147-52. https://doi.org/10.1016/S0140-6736(10)61972-1*

19. *Brossier F, Veziris N, Aubry A, Jarlier V, Sougakoff W. Detection by genotype MTBDRsl test of complex mechanisms of resistance to second-line drugs and ethambutol in multidrug-resistant Mycobacterium tuberculosis complex isolates. J Clin Microbiol 2010;48:1683-9. https://doi.org/10.1128/JCM.01947-09*

20. *Kim S, Kim Y, Chang Y, Hirgo WK, Chang CL, Shim TS, et al. Comparison of quantamatrix multiplexed assay platform and GenoType MTBDR assay using smear-positive sputum specimens from patients with multidrug-resistant/extensively drug-resistant tuberculosis in South Korea. Frontiers Microbiol 2019;10:1075 https://doi.org/10.3389/fmicb.2019.01075*

21. *Zhang Y, Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis: Update. Int J Tuberc Lung Dis 2015;19:1276-89. https://doi.org/10.5588/ijtld.15.0389*

22. *Gönlügür T, Gönlügür U. INH direnç mekanizmaları. Dicle Tıp Derg 2017;44:287-92. https://doi.org/10.5798/dicletip.339020*

23. *Oudghiri A, Karimi H, Chetioui F, Zakham F, Bourkadi JE, Elmessaoudi MD. Molecular characterization of mutations associated with resistance to second-line tuberculosis drug among multidrug-resistant tuberculosis patients from high prevalence tuberculosis city in Morocco. BMC Infect Dis 2018;18:1-8. https://doi.org/10.1186/s12879-018-3009-9*

24. *Gupta A, Sinha P, Nema V, Gupta PK, Chakraborty P, Kulkarni S, et al. Detection of Beijing strains of MDR M. tuberculosis and their association with drug resistance mutations in kat G, rpo B, and emb B genes. BMC Infect Dis 2020;20:1-7. https://doi.org/10.1186/s12879-020-05479-5*

25. *Avalos E, Catanzaro D, Catanzaro A, Ganiats T, Brodine S, Alcaraz J, et al. Frequency and geographic distribution of gyrA and gyrB mutations associated with fluoroquinolone resistance in clinical Mycobacterium tuberculosis isolates: A systematic review. Plos One 2015:10. https://doi.org/10.1371/journal.pone.0120470*

26. *Nosova EY, Bukatina AA, Isaeva YD, Makarova MV, Galkina KY, Moroz AM. Analysis of mutations in the gyrA and gyrB genes and their association with the resistance of Mycobacterium tuberculosis to levofloxacin, moxifloxacin and gatifloxacin. J Med Microbiol 2013;62:108-13. https://doi.org/10.1099/jmm.0.046821-0*

27. *Pantel A, Petrella S, Veziris N, Brossier F, Bastian S, Jarlier V, et al. Extending the definition of the GyrB quinolone resistance-determining region in Mycobacterium tuberculosis DNA gyrase for assessing fluoroquinolone resistance in M. tuberculosis. Antimicrob Agents Chemother 2012;56:1990-6. https://doi.org/10.1128/AAC.06272-11*

28. *Sullivan EA, Palumbo L, Ebrahimzadeh A, Frieden TR, Kreiswirth BN, Kapur V, et al. Emergence of fluoroquinolone-resistant tuberculosis in New York City. The Lancet 1995;345:1148-50. https://doi.org/10.1016/S0140-6736(95)90980-X*

29. *Mokrousov I, Otten T, Manicheva O, Potapova Y, Vishnevsky B, Narvskaya O, et al. Molecular characterization of ofloxacin-resistant Mycobacterium tuberculosis strains from Russia. Antimicrob Agents Chemother 2008;52:2937-9. https://doi.org/10.1128/AAC.00036-08*

30. *Zhu C, Zhang Y, Shen Y, Siu GK, Wu W, Qian X, et al. Molecular characterization of fluoroquinolone-resistant Mycobacterium tuberculosis clinical isolates from Shanghai, China. Diagn Microbiol Infect Dis 2012;73:260-3. https://doi.org/10.1016/j.diagmicrobio.2012.03.025*

31. *Disratthakit A, Prammananan T, Tribuddharat C, Thaipisuttikul I, Doi N, Leechawengwongs M, et al. Role of gyrB mutations in pre-extensively and extensively drug-resistant tuberculosis in Thai clinical isolates. Antimicrob Agents Chemother 2016;60:5189-97. https://doi.org/10.1128/AAC.00539-16*

32. *Singhal R, Reynolds PR, Marola JL, Epperson LE, Arora J, Sarin R, et al. Sequence analysis of fluoroquinolone resistance-associated genes gyrA and gyrB in clinical Mycobacterium tuberculosis isolates from patients suspected of having multidrug-resistant tuberculosis in New Delhi, India. J Clin Microbiol 2016;54:2298-305. https://doi.org/10.1128/JCM.00670-16*

33. *Chaoui I, Oudghiri A, El Mzibri M. Characterization of gyrA and gyrB mutations associated with fluoroquinolone resistance in Mycobacterium tuberculosis isolates from Morocco. J Glob Antimicrob Resist 2018;12:171-4. https://doi.org/10.1016/j.jgar.2017.10.003*

34. *Li Q, Gao H, Zhang Z, Tian Y, Liu T, Wang Y, et al. Mutation and transmission profiles of second-line drug resistance in clinical isolates of drug-resistant Mycobacterium tuberculosis from Hebei Province, China. Front Microbiol 2019;10:1838. https://doi.org/10.3389/fmicb.2019.01838*

35. *Ramakrishna V, Singh PK, Prakash S, Jain A. Second line injectable drug resistance and associated genetic mutations in newly diagnosed cases of multidrug-resistant tuberculosis. Microbial Drug Resistance 2020;26:971-5. https://doi.org/10.1089/mdr.2019.0215*

36. *Kateete DP, Kamulegeya R, Kigozi E, Katabazi FA, Lukoye D, Sebit SI, et al. Frequency and patterns of second-line resistance conferring mutations among MDR-TB isolates resistant to a second-line drug from eSwatini, Somalia and Uganda (2014-2016). BMC Pulm Med 2019;19:124. https://doi.org/10.1186/s12890-019-0891-x*

37. *Jugheli L, Bzekalava N, Fissette K, Portaels F, Rigouts L. High level of cross-resistance between kanamycin, amikacin, and capreomycin among Mycobacterium tuberculosis isolates from Georgia and a close relation with mutations in the rrs gene. Antimicrob Agents Chemother 2009;53:5064-8. https://doi.org/10.1128/AAC.00851-09*

38. *Evans J, Segal H. Novel multiplex allele-specific PCR assays for the detection of resistance to second-line drugs in Mycobacterium tuberculosis. J Antimicrob Chemother 2010;65:897-900 https://doi.org/10.1093/jac/dkq047*

39. *Yuan X, Zhang T, Kawakami K, Zhu J, Li H, Lei J, Tu S. Molecular characterization of multidrug-and extensively drug-resistant Mycobacterium tuberculosis strains in Jiangxi, China. J Clin Microbiol 2012;50:2404-13. https://doi.org/10.1128/JCM.06860-11*

Address for Correspondence/Yazışma Adresi

Dr. Gülnur TARHAN

Department of Medical Microbiology,
Adıyaman University Faculty of Medicine,
Adıyaman-Türkiye

E-posta: gulnur.tarhan@yahoo.com