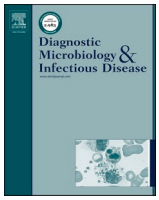
Diagnostic Microbiology & Infectious Disease 112 (2025) 116865



Contents lists available at ScienceDirect

Diagnostic Microbiology & Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio



Original Article

Performance of a molecular beacon-based qPCR method for detection of fluoroquinolone resistance in Mycobacterium tuberculosis: A study confirmed by sequencing

Oguz Arı [a], Rıza Durmaz [b,*], Ahmet Arslanturk [c]

[a] Central Research and Application Center, Ankara Yildirim Beyazit University, Ankara, Turkey
[b] Department of Clinical Microbiology, Faculty of Medicine, Ankara Yildirim Beyazit University, Ankara, Turkey
[c] National Tuberculosis Reference Laboratory, Department of Microbiology Reference Laboratories and Biological Products, General Directorate of Public Health, Ministry of Health, Ankara, Turkey

ARTICLE INFO

Keywords:
Drug resistance
Fluoroquinolone
Mutations
Mycobacterium tuberculosis
qPCR

ABSTRACT

Multidrug-resistant tuberculosis (MDR-TB) poses a serious threat to the control and treatment of tuberculosis (TB). Rapid diagnosis of resistant strains via utilization of molecular techniques is of critical importance on the proper management of the patients. The objective of this study was to develop a quantitative polymerase chain reaction (qPCR) method for the detection of mutation-based fluoroquinolone (FQ) resistance in Mycobacterium tuberculosis isolates. Primers and a molecular-beacon probe were designed to target quinolone-resistance-determining-region (QRDR) of the *gyr*A gene of *M. tuberculosis*. Amplification conditions and concentrations of primers/probe were optimized, and the effectiveness of the optimized qPCR method was tested on 50 MDR *M. tuberculosis* strains. To confirm the qPCR results, all strains were also screened for mutations in the *gyr*A gene using Sanger sequencing. The optimized qPCR method had analytical sensitivity of 96.5 cfu/ml. The method detected FQ resistance in 7 (14 %) of the 50 MDR-strains via either no or significantly decreased fluorescence signal due to mutations associated with FQ resistance. The sequencing of these seven strains detected three resistance-associated mutations (A90V, D94A and D94G). Of the 43 FQ-susceptible strains, 10 strains with wild-type gene sequences yielded strong fluorescent signals above 450 RFU, while the remaining 33 strains harboring a non-resistance-associated mutation (S95T) showed decreased fluorescence signals <350 RFU. The sensitivity and specificity of the qPCR method for detection of the mutations related to resistance was ≥99.9 % and 98 %, respectively. Consequently, the utility of the optimized qPCR method for the identification of mutation-based FQ resistance is promising.

1. Introduction

Tuberculosis (TB) is one of the major global health challenges and its prevalence increased in the last three years. According to the latest Global Tuberculosis Report of World Health Organization in 2024, the estimated new cases were 10.8 million in 2023, 10.7 million in 2022, and 10.4 million in 2021 [1]. Among TB cases, the rise of multidrug-resistant tuberculosis (MDR-TB), with an estimated 400,000 global cases in 2023, remains a significant public health concern, particularly in developing countries. Within MDR-TB cases, fluoroquinolone (FQ) resistance rate was reported as 19 % [1]. According to the latest tuberculosis report published in 2023, there were approximately 9,000 TB cases in Turkey in 2021. The rate of MDR-TB was 2.2 % in new cases ($n = 88$), while it was 8.6 % in previously treated cases ($n = 25$). Among these, three cases were reported as extensively drug-resistant (XDR-TB) [2].

The treatment of TB has become increasingly challenging due to the emergence of MDR- and XDR-*M. tuberculosis* (MTB) strains [3]. MDR-MTB strains are characterized by resistance to both rifampicin and isoniazid. MDR strains which are resistant to at least one FQ are classified as pre-XDR, while those exhibiting additional resistance to a parenteral drug are classified as XDR [4]. Poor management of patients with MDR-MTB strains contributes to the emergence of XDR *M. tuberculosis* strains [5]. The treatment of XDR-TB is more challenging compared to MDR-TB [6]. The management of XDR-TB requires better resources and higher financial support, leading to a higher prevalence,

* Corresponding author at: Department of Clinical Microbiology, Faculty of Medicine, Ankara Yildirim Beyazit University, Ankara, Turkey.
E-mail address: rdurmaz@aybu.edu.tr (R. Durmaz).

https://doi.org/10.1016/j.diagmicrobio.2025.116865
Received 6 March 2025; Received in revised form 16 April 2025; Accepted 19 April 2025
Available online 22 April 2025
0732-8893/© 2025 Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

particularly in low-income communities [7].

FQ antibiotics inhibit the topoisomerase II (DNA gyrase) enzyme, thereby interfering with mycobacterial DNA replication and transcription [8]. Mutations in the QRDR of *gyr*A gene such as A90V, S91P, D94A, D94G, D94H, D94N, D94Y and *gyr*B gene such as codons 500 and 538 mutations, which encode the DNA gyrase enzyme have been associated with FQ resistance [9,10]. Mutations in *gyr*A gene cause high-level resistance, while mutations in *gyr*B gene are responsible for low-level resistance [10].

Decline in the TB incidence and its control can be achieved by early diagnosis, accurate treatment of TB cases, and development of a more effective vaccine. By the tremendous improvement in technology, in addition to known conventional methods, several novel approaches such as quantitative polymerase chain reaction (qPCR) and artificial intelligence have become popular for diagnosis, prediction of drug resistance, and monitoring response to anti-tuberculosis treatment [11,12]. Additionally, similar to many infectious disease, several modern technology based vaccines such as mRNA vaccines and nano vaccines have been tested to develop more effective TB vaccines [13,14]. Even though all these attempts, there is no licensed TB vaccine, which is more effective than the Bacillus Calmette–Guérin (BCG), yet. Therefore, at the current time, qPCR methods have become of critical importance on the early diagnosis and management of TB cases. Molecular beacon probes, which can be used in qPCR assays, bind with high specificity to the target sequence through their loop region. While the 3′ end of the probe is labeled with a fluorescent dye, the 5′ end contains a quencher that suppresses fluorescence. If there is a variation in the target sequence that prevents binding, the probe structure remains intact, and fluorescence continues to be suppressed [15]. This method has the capability to detect any mutations in the QRDR region of the *gyrA* gene associated with FQ resistance.

This study aimed to develop a molecular beacon probe-based qPCR assay to detect mutation-based FQ resistance among *M. tuberculosis* strains. Additionally, the DNA sequencing method was used to confirm the qPCR results.

2. Materials and Methods

2.1. Design

In this study, a molecular beacon-based qPCR method was designed to detect the mutations responsible for high-level FQ resistance in *M. tuberculosis* isolates. For this purpose, a primer pair to amplify and a CY5.5-labeled molecular beacon probe, which was complementary to wild-type codons from 89 to 95 in QRDR region of the *gyrA* gene were designed. In this multiplex PCR mix, an additional primer pair specific to the *M. tuberculosis* IS*6110* gene and a FAM-labeled probe were also included as an internal control (Table 1). In cases if a mutation is present in the probe-binding part of the QRDR region in the *gyr*A gene, fluorescence signal will be detected from the internal control channel (FAM) during the PCR amplification phase, while no fluorescence signal will be expected from the CY5.5 channel. If no mutation is present in the probe-binding site in the QRDR region, signals will be detected from both channels.

Table 1
Sequences of primers and probe used in the PCR method.

Gene	Sequence
Internal Control (IS*6110*)	Forward: 5-CTAACCGGCTGTGGGTAG-3 Reverse: 5-GTCTTTCAGGTCGAGTAC-3 Probe: 5′-FAM-GCACCGAGGTGGCCATCGTGGAAGCGGGTGC-BHQ1-3′
gyrA	Forward: TTGCCGAGACCATGGGCAACTA Reverse: GAGCCGAAGTTGCCCTGG Probe: 5′-CY5.5-CCCGTTGACGCGTCGATCTACGACAGCAACGGG-BHQ3-3′

Note: Stem portion of the probes were underlined.

2.2. Samples and optimization of qPCR parameters

This retrospective study was performed on DNA samples isolated from a control strain (*M. tuberculosis* H37Ra) along with 50 MDR-*M. tuberculosis* clinical isolates. MDR-*M. tuberculosis* isolates were randomly selected among the *M. tuberculosis* clinical isolates previously identified in National Tuberculosis Reference Laboratory, Ministry of Health, Ankara, Turkey in 2023. The antibiotic resistance of the strains was determined using the GenoType MTBDRplus ver 2.0 (Bruker, USA) assay. *M. tuberculosis* H37Ra strain was used as a positive control and reference sequence in all experiments. Ethical approval of the study was obtained from the Ethics Committee of the Ankara Bilkent City Hospital (No: E2-24-6153). qPCR assays were performed with a total reaction volume of 20 µl, consisting of 10 µl of 2x Premix Ex master mix (Takara, Japan), 5 µl of primer/probe mix, and 5 µl of template DNA. All PCR reactions were performed using the CFX96 Real-Time PCR system (Biorad, USA). To determine the optimal amplification parameters, DNA amplification was done at different annealing temperatures (54°C, 56°C, 58°C and 60°C). The initial denaturation was performed at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 20 s, gradient annealing at 54/56/58/60°C for 20 s, and extension at 72°C for 20 s. Florescence signal was detected at the annealing step. To detect the optimal primer and probe concentrations, reactions with primer concentrations ranging from 0.2 to 0.6 pmol/µl and probe concentrations ranging from 0.1 to 0.4 pmol/µl were performed. In these experiments, the conditions were optimized to achieve an average fluorescence signal of 1000 RFU on the *M tuberculosis* H37Ra.

2.3. Analytical sensitivity/Limit of Detection (LoD) Analysis

LoD of the method was detected by DNA samples isolated from TB negative sputum samples spiked with 1000, 500, 250, 125, 100, 50 cfu/mL of *M. tuberculosis* H37Ra culture suspensions. After homogenization and decontamination of spiked sputum samples with the N-acetyl-l-cysteine-Sodium hydroxide method, DNA extraction was performed from each dilution using the QIAmp DNA Mini Kit (Qiagen, Germany). Purified DNA products from each dilution were used in qPCR assays in eight replicates on three separate days. The positivity rates of each dilution were used in Probit analysis to calculate LoD value of the method in 95 % hit rate.

2.4. DNA sequencing of gyrA gene

In order to confirm the qPCR results, DNA sequencing of QRDR in the *gyrA* gene of the 50 MDR-MTB strains was performed using the Sanger sequencing method. PCR assays were conducted by the same primers used in qPCR studies following amplification conditions described above. PCR products were purified using ExoSAP-IT (Applied Biosystems, Santa Clara, USA) kit and sequenced on ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, California). Cycle-sequencing reaction was performed with reverse primer using the Big-Dye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, USA) according to the manufacturer's instructions. The raw sequencing data were analyzed using the Ape Plasmid Editor (v3.1.7) software.

3. Results

3.1. Optimization of qPCR parameters

The optimal concentrations for the *gyr*A-targeting primer/probe in the qPCR assay were determined as 0.6 pmol/µl for the primers and 0.4 pmol/µl for the probe. A temperature gradient study was conducted to determine the optimal annealing temperature, and the highest fluorescence signal was observed at 60°C (Figure 1). The amplification

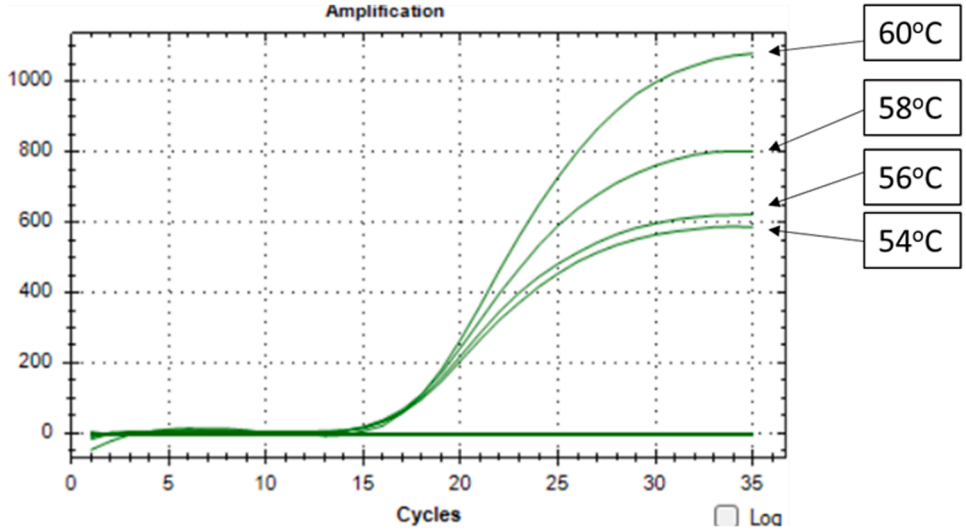


Figure 1. Fluorescent signals obtained from DNA extracted from *M. tuberculosis* H37Ra strain at different annealing temperatures.

parameters for the optimized qPCR method were defined as an initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 20 s, annealing at 60°C for, and extension at 72°C for 20 s.

3.2. Analytical sensitivity/Limit of Detection Analysis

Based on probit regression analysis, the limit of detection (LoD) for the optimized qPCR method was determined as 96.3 CFU/mL (95 % CI: 82.2–129.2) (Figure 2). Thus, the qPCR method can reliably detect *M. tuberculosis* at 96.3 copies/mL with 95 % probability.

3.3. Testing of M. tuberculosis strains with the optimized qPCR method

The *M. tuberculosis* H37Ra strain yielded fluorescent signal of approximately 1000 RFU. Of the 50 MDR-MTB strains, 10 had fluorescent signals above 450 RFU, 33 had fluorescence signals <350 RFU, and seven had no signals or signals <100 RFU (Figure 3). According to the qPCR results, the seven strains were considered FQ resistant, while the remaining 43 strains were FQ susceptible.

3.4. DNA sequencing of gyrA gene

The sequencing of the *gyr*A gene revealed that seven of the 50 MDR-MTB strains harbored three distinct mutations responsible for FQ resistance (Figure 4). Two of these mutations (D94A and D94G) were identified at codon 94 position. Each was observed on three different strains. The total rate of mutation at codon 94 was 85.7 % (6/7 strains). Subsequently, A90V mutation was identified as the third mutation in one FQ-resistant strain (14.3 %). Furthermore, all these seven FQ-resistant strains had also S95T variation. The remaining 43 MDR-MTB strains did not have mutation related to FQ resistance, however 33 of them had the S95T variation, which is not associated with FQ resistance.

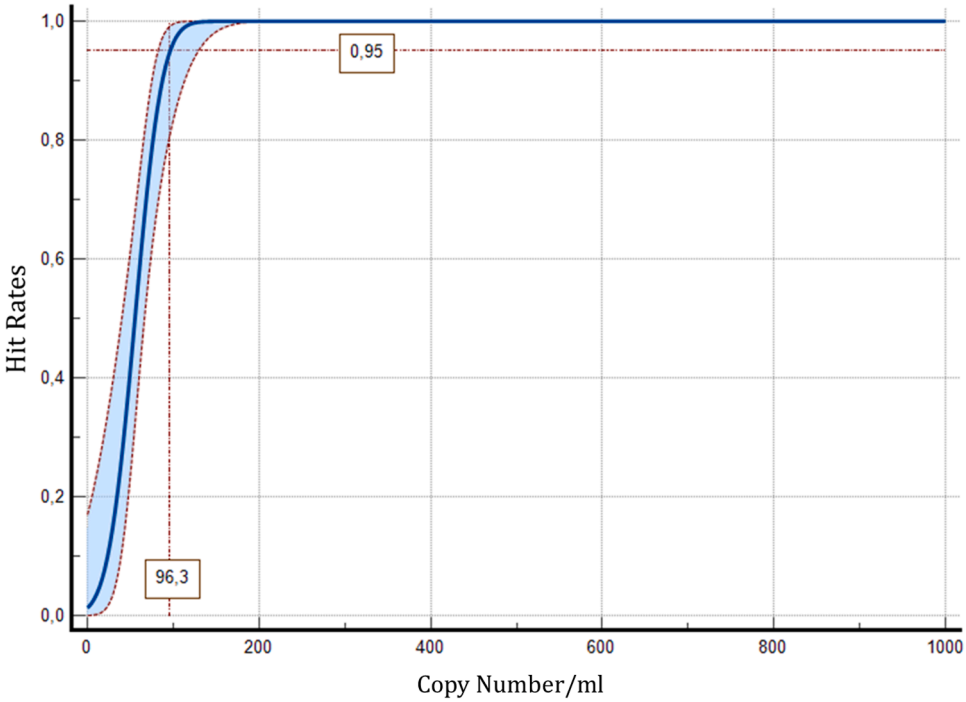


Figure 2. Plot of probit regression analysis regarding LoD of the qPCR method including 95 % confidence intervals.

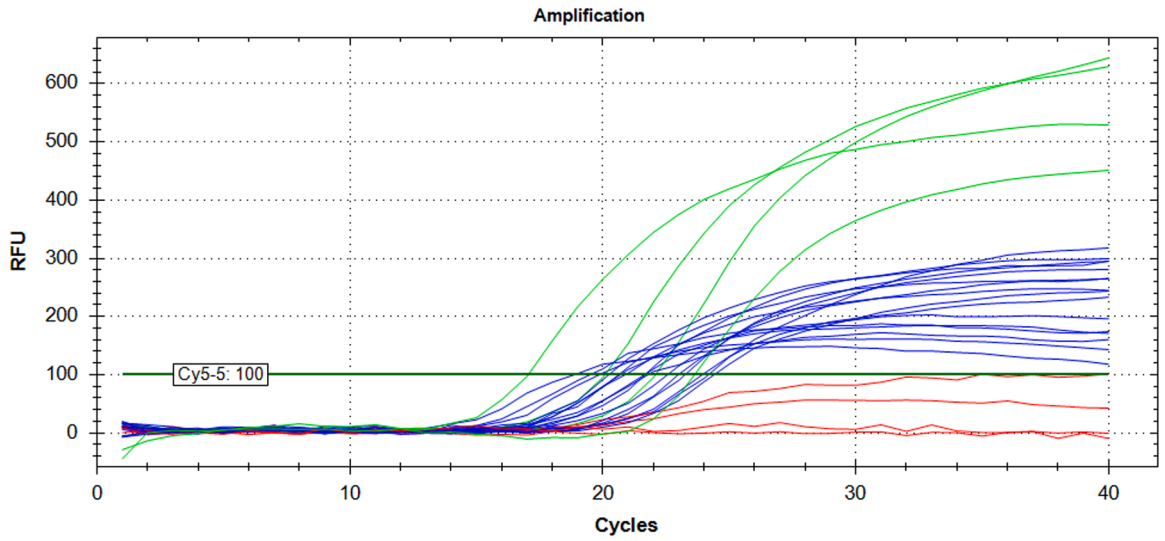


Figure 3. Examples of qPCR signals obtained from clinical samples of 4 resistant (Red), 4 wildtype (Green) and 15 susceptible strains with S95T variation (Blue). Threshold level adjusted at 100 RFU (Dark Green).

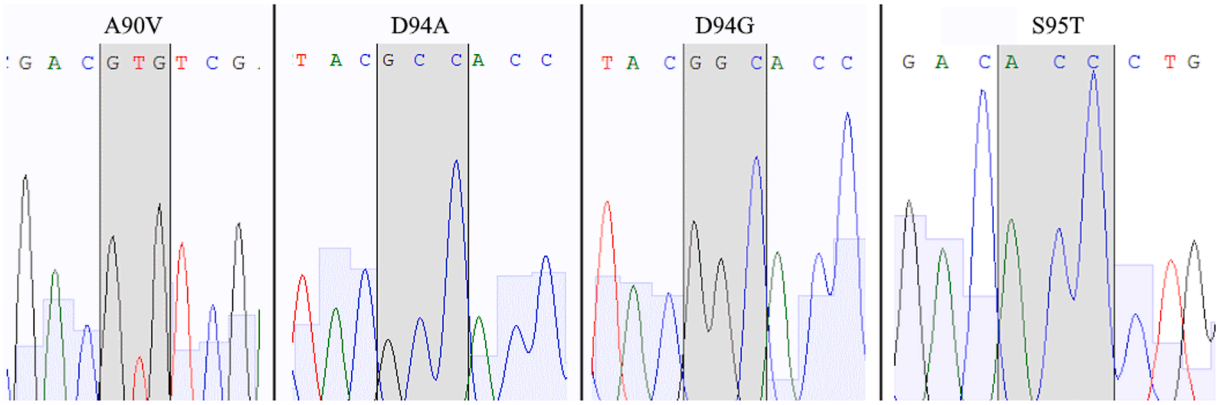


Figure 4. Electropherograms of *gyr*A mutations identified in *M. tuberculosis* strains. Codons containing mutations were highlighted in grey.

As shown in Table 2, seven resistant strains harbored both mutations D94G/A and S95T; however, susceptible strains had no mutation or had only S95T mutation. According to sequencing results, seven (14 %) of the 50 MDR-MTB strains were confirmed FQ resistant, the remaining 43 strains were FQ susceptible. Examples for QRDR sequences of wild-type strains ($n = 4$), FQ-susceptible strains with S95T mutation ($n = 5$), and FQ-resistant strains ($n = 7$) were shown in Figure 5.

3.5. Comparing qPCR results to those of DNA sequence analysis

Of the 43 FQ-susceptible strains, the 10 strains with wild-type *gyr*A sequences yielded fluorescent signals between 450 – 800 RFU, while the 33 strains harboring only S95T mutation resulted in fluorescent signals between 80 – 350 RFU. Due to the presence of the D94G, D94A, or A90V point mutations, the seven FQ-resistant strains had fluorescent signals <100 RFU. When the 100 RFU level was used as threshold, the mean cycle threshold (Ct) values of FQ-susceptible strains were 20.7 for wild type strains, and 22.2 for those with S95T mutation. On the other hand, resistant strains yielded no Ct value due to fluorescent signals below the threshold level, except one sample which passed the threshold at a very late cycle (39.6) (Table 3).

According to the comparison of results obtained from qPCR method and DNA sequencing, the clinical sensitivity and specificity of the developed qPCR method were 99.9 % and 98 %, respectively (Table 4).

4. Discussion

The increasing rate of drug-resistant TB cases has highlighted the critical importance of antimicrobial susceptibility testing [16]. In recent years, there has been a significant rise in the use of molecular tests for TB diagnosis and detection of drug resistance, which is generally linked to mutations or variations in specific genes [17,18]. Several commercial kits are currently available for the detection of FQ resistance and the diagnosis of XDR-TB. Among these, the Xpert MTB/XDR (Cepheid, USA) and GenoType MTBDR (Bruker, USA) kits are widely used. These kits have been endorsed by the WHO, validated in numerous laboratories and their findings have been published [19,20]. However, they also have some disadvantages including high costs due to single-use cartridges and the need for specialized instruments. Furthermore, isothermal amplification-based methods have also been described in the literature for the detection of *gyr*A mutations [21].

In the current study, a molecular beacon probe-based qPCR method was optimized to detect mutations responsible for FQ resistance, which is crucial for differentiating Pre-XDR *M. tuberculosis* strains. This method targets *gyr*A gene region known to harbor high-level FQ resistance-conferring mutations by using probe complementary to the wild-type sequence. In the presence of a mutation within the target region, the probe is unable to bind, consequently resulting in the absence of a fluorescence signal. This is then interpreted as resistance to the relevant drug.

Table 2
List of clinal samples and mutations identified in *gyr*A gene.

Sample Number	Resistance Profile	*gyr*A mutation associated FQ-resistant	*gyr*A mutation not associated FQ-resistant
1	FQ-Resistant	D94A	S95T
2	FQ-Resistant	D94A	S95T
3	FQ-Resistant	D94A	S95T
4	FQ-Resistant	D94G	S95T
5	FQ-Resistant	D94G	S95T
6	FQ-Resistant	D94G	S95T
7	FQ-Resistant	A90V	S95T
8	FQ-Susceptible	-	S95T
9	FQ-Susceptible	-	S95T
10	FQ-Susceptible	-	S95T
11	FQ-Susceptible	-	S95T
12	FQ-Susceptible	-	S95T
13	FQ-Susceptible	-	S95T
14	FQ-Susceptible	-	S95T
15	FQ-Susceptible	-	S95T
16	FQ-Susceptible	-	S95T
17	FQ-Susceptible	-	S95T
18	FQ-Susceptible	-	S95T
19	FQ-Susceptible	-	S95T
20	FQ-Susceptible	-	S95T
21	FQ-Susceptible	-	S95T
22	FQ-Susceptible	-	S95T
23	FQ-Susceptible	-	S95T
24	FQ-Susceptible	-	S95T
25	FQ-Susceptible	-	S95T
26	FQ-Susceptible	-	S95T
27	FQ-Susceptible	-	S95T
28	FQ-Susceptible	-	S95T
29	FQ-Susceptible	-	S95T
30	FQ-Susceptible	-	S95T
31	FQ-Susceptible	-	S95T
32	FQ-Susceptible	-	S95T
33	FQ-Susceptible	-	S95T
34	FQ-Susceptible	-	S95T
35	FQ-Susceptible	-	S95T
36	FQ-Susceptible	-	S95T
37	FQ-Susceptible	-	S95T
38	FQ-Susceptible	-	S95T
39	FQ-Susceptible	-	S95T
40	FQ-Susceptible	-	S95T
41	FQ-Susceptible	-	-
42	FQ-Susceptible	-	-
43	FQ-Susceptible	-	-
44	FQ-Susceptible	-	-
45	FQ-Susceptible	-	-
46	FQ-Susceptible	-	-
47	FQ-Susceptible	-	-
48	FQ-Susceptible	-	-
49	FQ-Susceptible	-	-
50	FQ-Susceptible	-	-

We observed the variations in qPCR fluorescent signals based on the presence or absence of mutations in the *gyr*A gene. The FQ-resistant strains harboring both the S95T and D94G/ D94A or A90V mutations yielded the lowest fluorescent signals, reflecting the impact of double mutations on probe binding site. The wild-type *M. tuberculosis* H37Ra strain and MDR-MTB strains with no mutation showed the highest fluorescence signals. Consequently, FQ-susceptible strains harboring S95T mutation alone, which does not confer FQ resistance [22], exhibited low fluorescent signals in comparison to the strains with wild-type *gyr*A gene sequence. The S95T mutation located within the probe-binding region interferes with probe binding, leading to reduced fluorescence signals <350 RFU. The level of the fluorescent signal in only one (3.3 %) of the 33 FQ-susceptible strains with S95T alteration was <100 RFU, which is in the range of some resistant strains. However, when 100 RFU value was used as a threshold, it was observed that all susceptible strains including the strains harboring S95T mutation had Ct values <27, except one strain, which generated no Ct due to low fluorescent signal (80 RFU). Although the rate of borderline results (RFU <100) is low (3.3 %) in our study, this low RFU value might result in confusion for interpretation of the results either FQ-resistant or susceptible. The threshold value of 100 RFU selected to differentiate between susceptible and resistant strains requires further validation with additional samples.

On the other hand, by using 100 RFU value as threshold, all FQ-resistant strains were detected via either generating no Ct or very late Ct value (≥39). Therefore, according to results of 43 FQ-susceptible and seven FQ-resistant strains, the optimized qPCR method showed promising performance, detecting FQ resistance with a sensitivity and a specificity of 99.9 % and 98 %, respectively. For comparison, a recent study using Xpert MTB/XDR kit reported that sensitivity of the kit was 91 % (95 CI, 81 %–97 %) while specificity was 100 % (95 CI, 98 %–100 %) for detection of FQ resistance [23].

The mutation screening of the *gyr*A gene expanded the approach of designing the qPCR method targeting the QRDR for FQ resistance. In the present study, three mutations (D94G, D94A and A90V) were identified in seven distinct FQ-resistant strains of the 50 MDR-MTB strains, indicating a FQ-resistance rate of 14 %. A recent study performed on 306 MDR-MTB strains in England showed that 23.9 % of the strains tested were resistant to FQ [24]. Among the seven FQ resistant strains in our study, the occurrence rate of D94G, D94A, and A90V mutations were 42.9 %, 42.9 % and 14.3 % respectively. Similar to our results, D94G and A90V mutations were reported as the predominant mutations with a

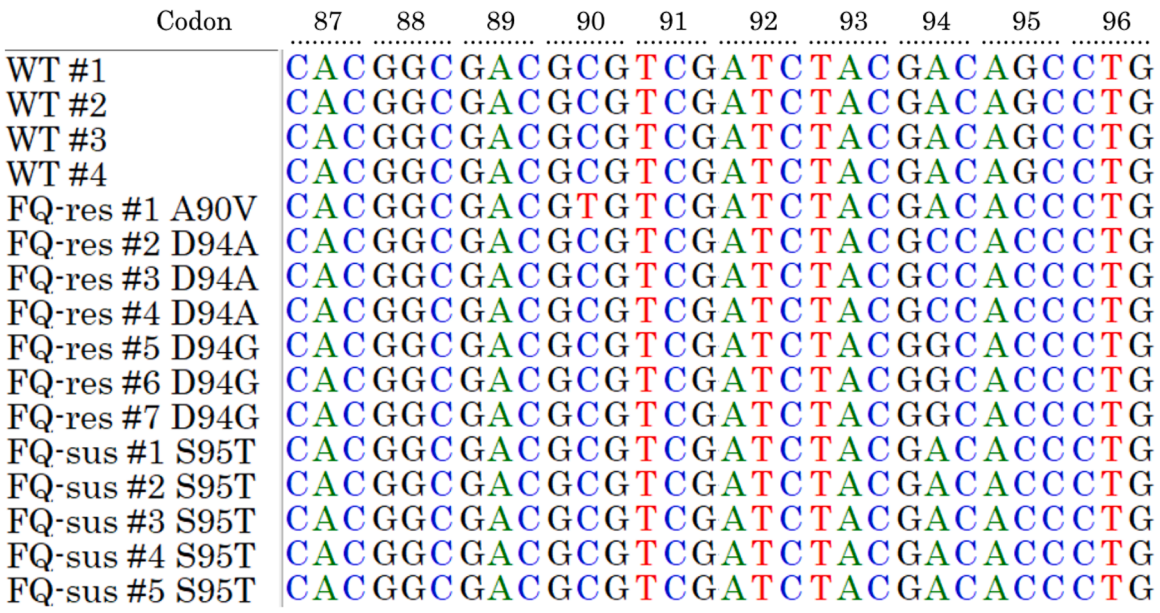


Figure 5. Examples for QRDR sequences of wild-type strains ($n = 4$), FQ-susceptible strains with S95T polymorphism ($n = 5$), and seven FQ-resistant strains.

Table 3
RFU and CT values of clinical samples tested with the developed qPCR method.

	Susceptible		Resistant
	Wild Type	S95T	
Mean RFU	700	281	25
Highest RFU	800	350	100
Lowest RFU	600	80	0
Mean CT	20.7	22.2	39.6
Highest CT	24.2	26.8	39.6
Lowest CT	17.1	18.9	39.6

Table 4
Specificity and sensitivity of qPCR method. *95 % CI.

	Sanger sequence (Reference Method)		
qPCR Method	FQ Susceptible	FQ Resistant	Total
FQ Susceptible	42	0	42
FQ Resistant	1	7	8
Total	43	7	50
Sensitivity (%)		100 (7/7) 59 - 100*	
Specificity (%)	98 (42/43) 88.7 - 99.9*		
Accuracy (%)	98 89.6 - 99.9*		

total observation rate of 80 % [25]. According to the WHO catalogue of mutation, the D94G alteration was the most prevalent (38.3 %) in case of FQ resistance [22]. There are limited numbers of studies on the identification of mutations in the *gyr*A gene of FQ-resistant *M. tuberculosis* in Turkey. A recent study performed on 37 MDR-MTB strains showed that 21.6 % of the strains were resistant to moxifloxacin and four distinct mutations (D94G, A90V, G88A, and D89N) were identified [26]. It is important to note that all of these mutations except codon 88 are also located within the binding range of the probe designed for our qPCR method. In contrast to the mutations observed in our samples, the most prevalent mutation identified in their isolates was G88A ($n = 3$), which was not detected in any of the samples included in our study. Another study investigating *gyr*A mutations in 14 FQ-resistant *M. tuberculosis* strains reported that D94G mutation was the most prevalent (35.7 %, 5/14), followed by the A90V mutation in three isolates (21.4 %) [27]. In accordance with the current literature, although limited number of FQ-resistant samples were studied, D94G mutation appears to be the most frequent *gyr*A mutation in FQ resistant *M. tuberculosis* strains in Turkey.

Notably, the S95T variation was observed both in FQ-resistant and susceptible strains. All FQ-resistant strains harbored the S95T variation (100 %) and this alteration was detected in 76.7 (33/43) of the FQ-susceptible strains. In a previous study, the S95T polymorphism was reported in 70.8 % and 56 % of the MDR- and moxifloxacin-resistant *M. tuberculosis* strains, respectively [26]. Similar to our results, in a current study the S95T mutation was observed in 100 % of the 11 FQ-resistant strains [28]. It is well known that amino acid at codon 95 is polymorphic and can be either serine or threonine according to the phylogeny of the *M. tuberculosis* strain, and strains with S95T alone do not express FQ resistance [29,30]. Even though the S95T variation alone does not result in FQ resistance, it might be possible that the S95T polymorphism could potentially induce mutations in other regions. More comprehensive studies based on whole genome sequencing data and clinical observations will be helpful to clarify this opinion.

In conclusion, the high sensitivity and specificity rates of the developed qPCR method are promising for its diagnostic value for detection FQ-resistances based on mutations in the QRDR of *gyrA* gene. These findings also highlight the importance of careful interpretation of qPCR results, particularly in cases where non-resistance-associated mutations may affect probe binding and reduce fluorescence signals. Further qPCR tests with a larger clinical sample pool are recommended to strengthen the reliability of the method in clinical settings. Alternatively, developing a new multiplex qPCR method in combination with another probe targeting both S and T variations at codon 95 or designing of a new probe that excludes codon 95 in the *gyr*A gene can be useful to exclude undesirable reduction in fluorescent signals obtained from the sensitive strains due to presence of S95T mutation.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Ankara Bilkent City Hospital (Date: 24/01/2024 No: E2-24-6153).

CRediT authorship contribution statement

Oguz Arı: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Rıza Durmaz:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Ahmet Arslanturk:** Resources, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This study was supported by Ankara Yıldırım Beyazıt University University Research Foundation, (Project ID: 2466) andand Health Institutes of Türkiye (Project Number: 16942).

References

[1] Global Tuberculosis Report 2024. 1st ed. Geneva: World Health Organization; 2024.
[2] Türkiye'de Verem Savaşı 2021 Raporu. Ankara: T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü; 2023.
[3] Chin KL, Anibarro L, Chang ZY, Palasuberniam P, Mustapha ZA, Sarmiento ME, et al. Impacts of MDR/XDR-TB on the global tuberculosis epidemic: challenges and opportunities. Curr Res Microb Sci 2024;7:100295. https://doi.org/10.1016/j.crmicr.2024.100295.
[4] Gupta M, Ish P, Malhotra N. Recent updates in diagnosis and management of drug-resistant tuberculosis in India: a paradigm shift and the way ahead during the COVID-19 crisis. Indian J Tuberc 2022;69:264–7. https://doi.org/10.1016/j.ijtb.2021.08.013.
[5] Matteelli A, Roggi A, Carvalho ACC. Extensively drug-resistant tuberculosis: epidemiology and management. Clin Epidemiol 2014:111. https://doi.org/10.2147/CLEP.S35839.
[6] Eker B, Ortmann J, Migliori GB, Sotgiu G, Muetterlein R, Centis R, et al. Multidrug- and extensively drug-resistant tuberculosis, Germany. Emerg Infect Dis 2008;14:1700–6. https://doi.org/10.3201/eid1411.080729.
[7] Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, Van Soolingen D, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. The Lancet 2010;375:1830–43. https://doi.org/10.1016/S0140-6736(10)60410-2.
[8] Espinosa-Pereiro J, Sánchez-Montalvá A, Aznar ML, Espiau M. MDR tuberculosis treatment. Medicina (Mex) 2022;58:188. https://doi.org/10.3390/medicina58020188.
[9] Chen J, Chen Z, Li Y, Xia W, Chen X, Chen T, et al. Characterization of *gyr*A and *gyr*B mutations and fluoroquinolone resistance in *mycobacterium tuberculosis* clinical isolates from Hubei Province, China. Braz J Infect Dis 2012;16:136–41. https://doi.org/10.1016/S1413-8670(12)70294-5.
[10] Nehru VJ, Jose Vandakunnel M, Brammacharry U, Ramachandra V, Pradhabane G, Mani BR, et al. Risk assessment and transmission of fluoroquinolone resistance in drug-resistant pulmonary tuberculosis: a retrospective genomic epidemiology study. Sci Rep 2024;14:19719. https://doi.org/10.1038/s41598-024-70535-y.
[11] Liang S, Ma J, Wang G, Shao J, Li J, Deng H, et al. The application of artificial intelligence in the diagnosis and drug resistance prediction of pulmonary tuberculosis. Front Med 2022;9:935080. https://doi.org/10.3389/fmed.2022.935080.
[12] Choudhary OP, Infant SS, As V, Chopra H, Manuta N. Exploring the potential and limitations of artificial intelligence in animal anatomy. Ann Anat - Anat Anz 2025;258:152366. https://doi.org/10.1016/j.aanat.2024.152366.
[13] Priyanka, Abusalah MAH, Chopra H, Sharma A, Mustafa SA, Choudhary OP, et al. Nanovaccines: a game changing approach in the fight against infectious diseases. Biomed Pharmacother 2023;167:115597. https://doi.org/10.1016/j.biopha.2023.115597.
[14] Priyanka, Chopra H, Choudhary OP. mRNA vaccines as an armor to combat the infectious diseases. Travel Med Infect Dis 2023;52:102550. https://doi.org/10.1016/j.tmaid.2023.102550.
[15] Huang K, Martí AA. Recent trends in molecular beacon design and applications. Anal Bioanal Chem 2012;402:3091–102. https://doi.org/10.1007/s00216-011-5570-6.
[16] Schön T, Miotto P, Köser CU, Viveiros M, Böttger E, Cambau E. *Mycobacterium tuberculosis* drug-resistance testing: challenges, recent developments and perspectives. Clin Microbiol Infect 2017;23:154–60. https://doi.org/10.1016/j.cmi.2016.10.022.
[17] Khawbung JL, Nath D, Chakraborty S. Drug resistant tuberculosis: a review. Comp Immunol Microbiol Infect Dis 2021;74:101574. https://doi.org/10.1016/j.cimid.2020.101574.
[18] Sanchini A, Lanni A, Giannoni F, Mustazzolu A. Exploring diagnostic methods for drug-resistant tuberculosis: a comprehensive overview. Tuberculosis 2024;148:102522. https://doi.org/10.1016/j.tube.2024.102522.
[19] Radhakrishnan R, Prabuseenivasan S, Hannah S, Vaishanavee V, Senthildevi V, Kannadhasan T, et al. Genotype MTBDRsl version 2 and phenotypic drug resistance detection of *mycobacterium tuberculosis* for fluoroquinolones and aminoglycosides. Int J Mycobacteriology 2023;12:299–304. https://doi.org/10.4103/ijmy.ijmy_120_23.
[20] Sethi S, Sharma S, Aggarwal AN, Dhatwalia SK, Rana R, Yadav R. Xpert MTB/XDR assay: rapid TB drug resistance detection. Infection 2024;52:2207–12. https://doi.org/10.1007/s15010-024-02260-7.
[21] Li M-C, Lu Y, Liu H-C, Lin S-Q, Qian C, Nan X-T, et al. Rapid detection of fluoroquinolone resistance in *mycobacterium tuberculosis* using a novel multienzyme isothermal rapid assay. J Antibiot (Tokyo) 2023;76:598–602. https://doi.org/10.1038/s41429-023-00639-6.
[22] Walker TM, Miotto P, Köser CU, Fowler PW, Knaggs J, Iqbal Z, et al. The 2021 WHO catalogue of *mycobacterium tuberculosis* complex mutations associated with drug resistance: a genotypic analysis. Lancet Microbe 2022;3:e265–73. https://doi.org/10.1016/S2666-5247(21)00301-3.
[23] Centner CM, Munir R, Tagliani E, Rieß F, Brown P, Hayes C, et al. Reflex Xpert MTB/XDR testing of residual rifampicin-resistant specimens: a clinical laboratory-based diagnostic accuracy and feasibility study in South Africa. Open Forum Infect Dis 2024;11:ofae437. https://doi.org/10.1093/ofid/ofae437.
[24] Ferran E, Chan C, Sheikh N, Dedicoat M, Alexander E, Gibertoni-Cruz A, et al. Population-level frequency of fluoroquinolone resistance by whole-genome sequencing drug predictions in *mycobacterium tuberculosis* complex isolates in England from 2017 through 2023. Clin Infect Dis 2025;80:660–2. https://doi.org/10.1093/cid/ciae560.
[25] Che Y, Song Q, Yang T, Ping G, Yu M. Fluoroquinolone resistance in multidrug-resistant *mycobacterium tuberculosis* independent of fluoroquinolone use. Eur Respir J 2017;50:1701633. https://doi.org/10.1183/13993003.01633-2017.
[26] Bozok T, Kayar B, Yakici G, Eker E, Marzi M, Karsli F, et al. Detection and Molecular analysis of moxifloxacin MIC values of *mycobacterium tuberculosis* strains isolated from clinical specimens. Mikrobiyol Bul 2019;53:245–53. https://doi.org/10.5578/mb.68291.
[27] Yazisiz H, Hircin Cenger D, Uçarman N, Altin S. The molecular patterns of resistance to anti-tuberculosis drugs: an analysis from Istanbul, Turkey. J Chemother 2020;32:66–74. https://doi.org/10.1080/1120009X.2020.1716477.
[28] Sayadi M, Zare H, Jamedar SA, Hashemy SI, Meshkat Z, Soleimanpour S, et al. Genotypic and phenotypic characterization of *mycobacterium tuberculosis* resistance against fluoroquinolones in the northeast of Iran. BMC Infect Dis 2020;20:390. https://doi.org/10.1186/s12879-020-05112-5.
[29] Mayer C, Takiff H. The Molecular genetics of fluoroquinolone resistance in *mycobacterium tuberculosis*. Microbiol Spectr 2014;2. https://doi.org/10.1128/microbiolspec.MGM2-0009-2013. 2.4.02.
[30] Maruri F, Sterling TR, Kaiga AW, Blackman A, Van Der Heijden YF, Mayer C, et al. A systematic review of gyrase mutations associated with fluoroquinolone-resistant *mycobacterium tuberculosis* and a proposed gyrase numbering system. J Antimicrob Chemother 2012;67:819–31. https://doi.org/10.1093/jac/dkr566.