Rapid screening of rpoB and katG mutations in Mycobacterium tuberculosis isolates by high-resolution melting curve analysis

Article *in* Indian Journal of Medical Microbiology · October 2014

DOI: 10.4103/0255-0857.142245

CITATIONS
7

READS
98

6 authors, including:

Mehri Haeili
University of Tabriz
28 PUBLICATIONS **315** CITATIONS
SEE PROFILE

Abbas Ali Imani Fooladi
257 PUBLICATIONS **2,149** CITATIONS
SEE PROFILE

Saeed Zakerbostanabad
PIAU University
239 PUBLICATIONS **397** CITATIONS
SEE PROFILE

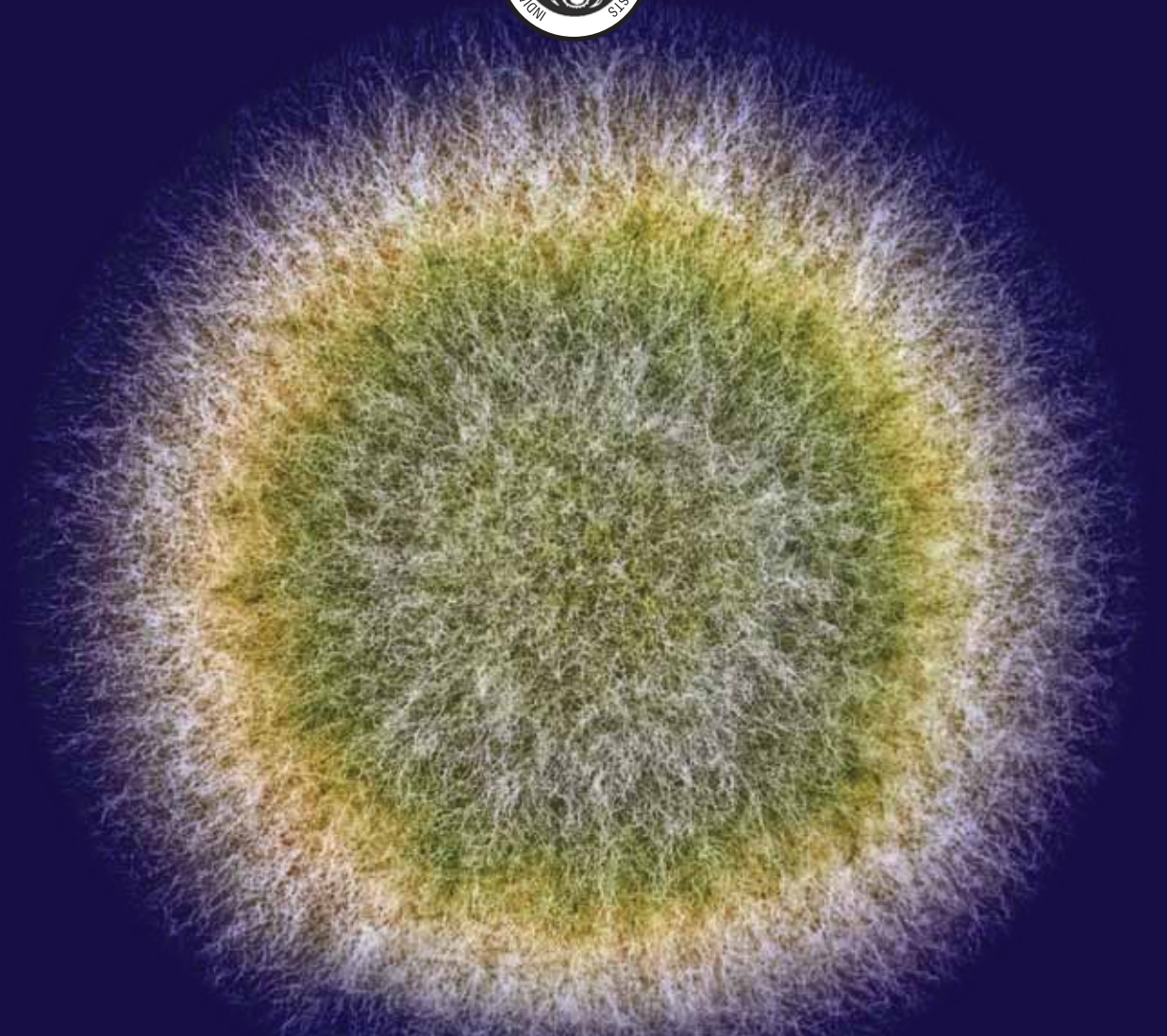
Davood Darban-Sarokhalil
Iran University of Medical Sciences
66 PUBLICATIONS **532** CITATIONS
SEE PROFILE

Impact Factor® for 2013: 1.037

ISSN 0255-0857

Volume 32 Number 4 October 2014

Indian Journal of Medical Microbiology



Publication of Indian Association of Medical Microbiologists

www.ijmm.org

Wolters Kluwer Health | Medknow

Indian Journal of Medical Microbiology, (2014) 32(4): 398-403

Original Article

Rapid screening of *rpoB* and *katG* mutations in *Mycobacterium tuberculosis* isolates by high-resolution melting curve analysis

M Haeili, AI Fooladi, SZ Bostanabad, DD Sarokhalil, F Siavoshi, *MM Feizabadi

Abstract

Background: Early detection of multidrug-resistant tuberculosis (MDR-TB) is essential to prevent its transmission in the community and initiate effective anti-TB treatment regimen. **Materials and Methods:** High-resolution melting curve (HRM) analysis was evaluated for rapid detection of resistance conferring mutations in *rpoB* and *katG* genes. We screened 95 *Mycobacterium tuberculosis* clinical isolates including 20 rifampin resistant (RIF-R), 21 isoniazid resistant (INH-R) and 54 fully susceptible (S) isolates determined by proportion method of drug susceptibility testing. Nineteen *M. tuberculosis* isolates with known drug susceptibility genotypes were used as references for the assay validation. The nucleotide sequences of the target regions *rpoB* and *katG* genes were determined to investigate the frequency and type of mutations and to confirm HRM results. **Results:** HRM analysis of a 129-bp fragment of *rpoB* allowed correct identification of 19 of the 20 phenotypically RIF-R and all RIF-S isolates. All INH-S isolates generated wild-type HRM curves and 18 out of 21 INH-R isolates harboured any mutation in 109-bp fragment of *katG* exhibited mutant type HRM curves. However, 1 RIF-R and 3 INH-R isolates were falsely identified as susceptible which were confirmed for having no mutation in their target regions by sequencing. The main mutations involved in RIF and INH resistance were found at codons *rpoB531* (60% of RIF-R isolates) and *katG*315 (85.7% of INH-R isolates), respectively. **Conclusion:** HRM was found to be a reliable, rapid and low cost method to characterise drug susceptibility of clinical TB isolates in resource-limited settings.

Key words: *High resolution melting curve analysis, HRM, Iran, katG, M. tuberculosis, ropB*

Introduction

The emergence of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* has become one of the most critical issues for tuberculosis (TB) control programmes worldwide. Establishment of rapid techniques enabling the prompt detection of drug resistance in clinical isolates is very crucial not only to reduce the time to effective treatment for MDR-TB patients but also to prevent further spread of drug resistant *M. tuberculosis* strains. However, conventional culture-based methods for drug susceptibility testing (DST) take several weeks before results become available, causing significant delay in identifying and properly treating MDR cases.

Drug resistance in *M. tuberculosis* is mostly conferred by mutations within the genes encoding drug targets or drug-converting enzymes.[1] Genetic studies have demonstrated that up to 98% of the resistance to rifampin (RIF) is associated with single-point mutations in the RNA polymerase gene *rpoB* comprising a defined region of 81-bp which is referred to as rifampin resistance-determining region (RRDR) (codons 507-533).[2] In contrast, resistance to isoniazid (INH) is linked to mutations occurring in one or more genes. But among them *katG* S315T mutation is the most common mutation, accounting for 50-95% of clinical resistance to INH.[3]

Iran with an estimated TB incidence rate of 17 per 100,000 shares geographical borders with three countries where TB is endemic, i.e., Pakistan, Iraq and Afghanistan.[4] A recent study from Iran reported that 2.8% of new TB cases and 51.9% of previously treated TB patients were MDR.[5] However, there is limited information about specific mutation patterns in drug resistant strains in Iran. Also, the lack of rapid, reliable and cost-effective molecular methods for DST may contribute to delayed

*Corresponding author (email: <mfeizabadi@tums.ac.ir>)
Department of Microbiology, School of Biology, College of Science, University of Tehran (MH, FS), [2]School of Medicine, Tehran University of Medical Sciences (MH, MMF), Applied Microbiology Research Center (AIF) Bagiyatallah University of Medical Sciences, Department of Mycobacteriology, (SZB) Massoud Laboratory, Tehran, School of Medicine, (MMF) Alborz University of Medical Sciences, Karaj, (DDS) Pediatric Infectious Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran
Received: 23-08-2013
Accepted: 13-02-2014

Access this article online	
Quick Response Code:	Website: www.ijmm.org
	DOI: 10.4103/0255-0857.142245

detection of MDR cases and therefore, their spread in this area. High-resolution melting curve analysis (HRM) is a recently developed technique for rapid and efficient mutation screening in nucleic acid samples. This post-PCR method requires only the usual unlabeled primers and a dsDNA binding dye and detects subtle genetic alterations in PCR-amplified samples based on their strand disassociation behavior.[6] It has been described that frequency and type of drug resistance conferring mutations in MDR strains can vary according to geographic regions.[7] This can affect the performance and reliability of molecular diagnostics in different area.[8,9] In the present study we aimed to evaluate HRM curve analysis for rapid identification of drug-resistant *M. tuberculosis* isolates and to determine the main mutations conferring resistance to INH and RIF in clinical isolates from Iran.

Materials and Methods

Bacterial strains and drug susceptibility testing

This study was conducted at the Department of Microbiology, Tehran University of Medical sciences, Tehran, Iran during 2010-2012. Two groups of Mycobacterial strains were included in this study. The first group of *M. tuberculosis* strains consisted of 4 RIF resistant (RIF-R), 5 INH resistant (INH-R) and 10 drug-susceptible (S) isolates which were used as reference samples. The genotype of these strains was previously determined by direct sequencing analysis and used for the initial assay development. The second group consisted of 10MDR, 10 RIF-mono-R and 11 INH-mono-R and 54 *M. tuberculosis* clinical isolates susceptible to both drugs as determined by phenotypic DST. The genotype of these isolates had not been determined previously (blind samples) and was subsequently identified by HRM and sequencing. All isolates were collected from hospitals in five different provinces of Iran including Tehran (n = 55), Alborz (n = 5), Sistan-Baluchestan (n = 15), Hormozgan (n = 15) and Kermanshah (n = 14) from 2009 to 2012. Drug susceptibility testing was performed using the proportion method on Lowenstein-Jensen (LJ) medium containing 0.2 µg/ml for INH or 40 µg/ml for RIF.[10] The *M. tuberculosis* H37Rv ATCC 27294 strain, susceptible to both RIF and INH, was included as an internal control.

DNA preparation and primer design

DNAs of *M. tuberculosis* strains were extracted as previously described [11]. All DNA samples were quantified by UV absorbance at 260 nm to ensure that the starting amount of template is similar between different samples. All primers utilised in this study were designed by using the Primer3Plus web tool (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/).

In order to detect mutations associated with RIF resistance, primers were designed to amplify a 129-bp fragment containing codons 507 to 536 of *rpoB* (*rpoB*-F, 5′- GCCGCGATCAAGGAGTTCTT; *rpoB*-R, 5′- ACGCTCACGTGACAGACCG).

For mutation analysis of *katG*, a 109 bp fragment including codon 315 was amplified using the following primers: *katG*-F, 5′-TGGGCTTGGGCTGGAAGA; *katG*-R, 5′- AAACTGTTGTCCCATTTCGTC.

PCR and high-resolution melting curve analysis

The mi-real-time EvaGreen® Master kit (Metabion, Martinsried, Germany) was used for PCR and HRM assay containing the following components per reaction mixture: 12.5 µl of 2X master mix, 1 µl of the template (50 ng/µl) and PCR grade water (Metabion) adjusted to a final volume of 25 µl. Primers were added to the appropriate master mix at concentrations of 100 nM *(rpoB)* or 200 nM *(katG)*. PCR, HRM and data analysis was performed on a Rotor-Gene 6000 apparatus (Qiagen) according to the method described by Ramirez *et al*.[12] with a minor modification. Briefly, PCR was performed under the following conditions: 1 cycle of 95°C for 2 min, 45 cycles of 95°C for 30s and 66°C (for *rpoB*) or 63°C (for *katG*) for 30s. The HRM analysis was performed following the amplifications by slow temperature increase from 75°C to 90°C for *katG* and 80°C to 95°C for *rpoB* at a rate of 0.1°C per step with continuous fluorescence detection. All samples were tested in duplicate. Rotor-Gene 6000 Series Software 1.7 was used for HRM analysis.

DNA sequencing

Sequencing was performed to determine the frequency and type of mutations and to confirm the results found by HRM. A 411-bp fragment of the *ropB* gene (including the 81-bp RRDR region) and 210-bp fragment of the *katG* (containing codon 315) gene were amplified using *rpoB*-F, 5′- TACGGTCGGCGAGCTGATCC; *rpoB*-R, 5′- TACGGCGTTTCGATGAACC and *katG*-F, 5′- GAAACAGCGGCGCTGATCGT; *katG*-R, 5′- GTTGTCCCATTTCGTCGGGG, respectively. The PCR mixture (50 µl) contained 1 × PCR reaction buffer, 200 µM dNTPs, 1.5 mM MgCl2 and 1 U of Taq polymerase, 300 nM of each primer and 2 µl of the DNA sample. The conditions of PCR amplification were 94°C for 10 min, followed by 35 cycles of 94°C for 30 s, 65°C (for *rpoB*) or 63°C (for *katG*) for 30 s, 72°C for 30 s, with a final extension at 72°C for 10 min. The PCR products were sent to Macrogen Company (Korea) for sequencing. Statistical analysis was performed using Chi-square test to determine statistical associations between mutation type and frequency and phenotypic DST and values of $P < 0.05$ were considered statistically significant.

Results

Genotypic analysis of RIF and INH resistance was

carried out using HRM assay on 104 clinical isolates of *M. tuberculosis*. To validate our assay, we tested 19 isolates with known genotype having two different mutations at *rpoB* [Ser531Leu, TCG → TTG (*n* = 3 isolates); Ser531Trp, TCG → TGG (*n* = 1 isolate)], 1 mutation at *katG* [Ser315Thr, AGC → ACC (*n* = 5 isolates)] and no mutation in both genes (*n* = 10 isolates). All reference resistant and susceptible isolates (group 1) were correctly identified as mutant and wild-type, respectively, by HRM assay. Figure 1 illustrates the normalised and difference graph of *rpoB* HRM profiles between reference wild-type and mutant strains.

Detection of rpoB mutations

The 129-bp fragment of *rpoB* gene was amplified from 20 RIF-R (10MDR and 10RIF- mono-R) and 54 RIF-S isolates (group 2). HRM analysis revealed two clusters of samples based on the shape of their melting curves [Figure 2a]. The first cluster comprised all RIF-S isolates including H37Rv and one RIF-mono-R isolate. Sequencing analysis confirmed that all these strains including the outlying RIF-R strain had indeed no mutation in the amplified region of *rpoB* gene. The second cluster of melting curves contained only samples from RIF-R isolates. These curves were clearly distinct from those of susceptible isolates. Therefore, we designated the second cluster as mutant type. Again these results were in full agreement with that of DNA sequencing. Taken together, 11 different mutations were observed in 81-bp core region of *rpoB*. The frequency of mutations at codons 531, 526 and 516 was 60% (*n* = 12/20), 5% (*n* = 1/20) and 0%, respectively, in all RIF-R isolates. A significant difference was observed for frequency of mutations at *rpoB531* between MDR (100%, *n* = 10 out of 10) and RIF-mono-R strains (20%, *n* = 2 out of 10) (*P*-value = 0.0014, Chi-square test). Moreover, one isolate had double mutations including Gln510Glu and His526Leu changes [Table 1].

Detection of katG mutations

HRM analysis of 109-bp fragment of *katG* correctly recognised mutations in 85.7% (*n* = 18/21) of phenotypically INH-R strains which, as for *rpoB*, showed a unique melting curve profile indistinguishable from each other but different from that of the other 3 INH-R (INH-mono-R) and all INH-S isolates [Figure 2b]. DNA sequencing confirmed that all 18 isolates distinguished by HRM, including 10 MDR and 8 INH- mono-R isolates harbored a Ser315Thr (AGC → ACC) mutation. All 54 INH-S and 3 INH-R strains which showed the wild-type melting pattern lacked any mutation in the target region of *katG*.

Discussion

Inexpensive and rapid diagnosis of drug resistant TB is crucial for the clinical management of tuberculosis especially in developing countries which are hit the hardest by the current TB pandemic. In the present study we utilised low-cost HRM analysis for rapid prediction of RIF and INH resistance. This is the first report of HRM application on clinically isolated *M. tuberculosis* strains from Iran. As expected, the HRM assay reliably predicted resistance to RIF and INH when resistance phenotypes were due to specific mutations in the respective *rpoB* and *katG* genes but failed to accurately determine drug resistance to RIF in one isolate and INH in three samples which were found repeatedly to be resistant by conventional DST. Nucleotide sequencing analysis confirmed the absence of mutations in the target regions of *katG* and *rpoB* in these samples. The absence of mutations in the hot spot regions of *rpoB* and *katG* in drug resistant isolates has been also reported in several other studies.[12,13] These discrepancies between phenotypic and genotypic drug susceptibility testing are likely attributable to mutations that occur outside the amplified *rpoB* or *katG* hot spot regions or that are located in different genes. For example, mutations in the promoter regions of the mabA/inhA operon or the ahpC gene have been demonstrated previously for INH-R isolates.[3] Also, resistance can be barely triggered by mutations outside the 81-bp hot spot region of *rpoB* gene.[14,15] A total of 95% of RIF-R isolates contained mutations in the 81-bp core region of *rpoB*. We found a significant difference in frequency of mutations in *rpoB*531 between the isolates with multiple-drug resistance (100%) and isolates with resistance only to RIF (20%) (*P* = 0.0014). This difference can be explained by the fact that mutations at this site provide higher selective pressure in MDR isolates.[8] This finding indicates that *rpoB*531 can be considered as a valuable marker for RIF resistance prediction in MDR cases in this geographic region. Furthermore, in our study codon 516 of the *rpoB* gene did not contribute to RIF resistance and mutations at codon 526 represented only 5% of resistance conferring mutations. Frequency of drug resistance conferring mutations may vary in drug-resistant *M. tuberculosis* isolates based on geographic regions. For instance, a study from New York and Texas showed 31%, 43% and 5% of RIF-R *M. tuberculosis* isolates carried mutations at codons 531, 526 and 516, respectively,[15] while these frequencies were 48.5%, 21.3%, 16.9% in a study from Korea[16] and 60%, 23%, 5% in a study from Pakistan.[17] Aslan and coworkers from Turkey reported that the most frequent mutations were found at codons 531 (64.2%), 545 (28.6%), 516 (7.1%) and 524 (7.1%) of *rpoB* gene region; however, no mutation was detected at *rpoB*526 codon.[18] One possible reason for this discrepancy is the use of different drug analogues in diverse geographic locations which provides a selective force for different mutations.[7]

Among the INH-R strains, 85.7% carried a mutation at *katG*315 (S315T). Similarly, 91.7%, 82.9% and 76.6% of INH-R isolates in Russia,[19] India[20] and Turkey,[18] respectively, have been reported for harbouring mutation at the

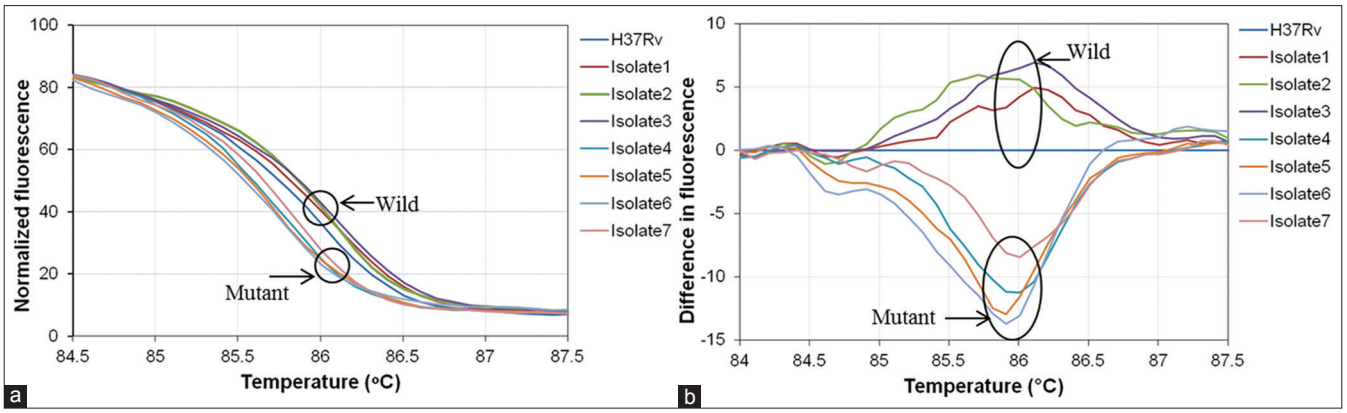


Figure 1: HRM normalised (a) and difference plot (b) for 129 bp fragment of *rpoB* gene in reference isolates. HRM curve of *M. tuberculosis* H37Rv was compared with those of other clinical isolates. Existence of mutation in amplicon has changed the shape of melting curve in mutant isolates

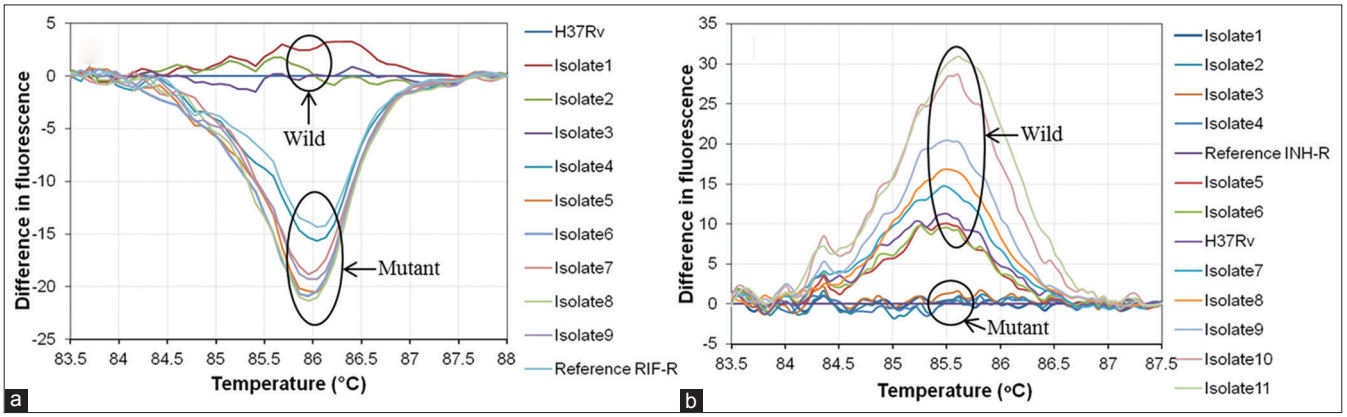


Figure 2: (a) HRM difference plot of *rpoB* in phenotypically RIF-R isolates. HRM curve of *M. tuberculosis* H37Rv isolate was compared with those of other samples. (b) HRM difference plot for 109-bp fragment of *katG* in phenotypically INH-R isolates. HRM curve of a genotypically determined INH-R isolate was compared with those of other samples

Table 1: Comparison of results obtained by HRM and DNA sequencing for detection of mutations conferring INH and RIF resistance in drug resistant and susceptible *M. tuberculosis* isolates

Phenotype	Gene	Amino acid change (s)	Nucleotide change (s)	No. of strains	HRM
RIF-R	*rpoB*	Ser531Trp	TCG→TGG	1	Mutant
	rpoB	Ser531Leu	TCG→TTG	11	Mutant
	rpoB	Leu533Pro	CTG→CCG	2	Mutant
	rpoB	Leu511Pro	CTG→CCG	1	Mutant
	rpoB	Thr508Pro	ACC→CCC	1	Mutant
	rpoB	Gly507Asp	GGC→GAC	1	Mutant
	rpoB	Gln510Glu, His526Leu	CAG→GAG, CAC→CTC	1	Mutant
	rpoB	Met515Ile	ATG→ATA	1	Mutant
	rpoB	-	-	1	Wild-type
INH-R	*katG*	Ser315Thr	AGC→ACC	18	Mutant
	katG	-	-	3	Wild-type
INH-S, RIF-S	*katG, rpoB*	Wild-type	-	54	Wild-type

RIF-R: Rifampin resistant, INH-R: Isoniazid resistant, HRM: High-resolution melting curve

same genetic location. We found that mutation frequency at *katG*315 in INH-mono-R isolates was lower (72.7%, $n = 8/10$) than that found in MDR strains (100%). However, this difference was not statistically significant ($P = 0.2$, Chi-square

test). Similar results were obtained by Hillemann *et al*.[21] who showed 100% of MDR and 90% of INH-mono-R isolates from Kazakhstan carried a mutation in codon 315 of the *katG* gene. In contrast, Bakonyte *et al*.[22] reported a significant difference in frequency of mutations between isolates resistant only to INH (50%) and MDR strains (88.5%).

Compared to phenotypic drug susceptibility tests, the HRM assay described here is characterised by 95% sensitivity and 100% specificity for detection of RIF resistance. INH resistance was detected with a sensitivity of 85.7% and specificity of 100%. Ramirez *et al*.[12] showed sensitivity and specificity to be 91% and 98% for RIF resistance, 87 and 100% for INH resistance. Moreover, a study from Austria[14] found the sensitivity and specificity of the HRM to be 95.9 and 100%, respectively, for RIF resistance detection. However, there is a large variation in sensitivity of molecular diagnostics because of the diverse frequency and type of mutations in different geographic regions.

Conclusions

In conclusion our results showed that 100% of MDR strains had mutations at *rpoB*531 and *katG*315. This finding indicates that these two codons can serve as good markers for rapid prediction of MDR-TB cases by HRM method in Iran. HRM analysis is a closed-tube, reliable, simple and low-cost molecular method which does not require expensive probes or other specialised reagents. It is, therefore, suitable for rapid screening of a large number of samples in developing settings. In addition, since the method is DNA based it does not require advanced safety protocols and can be conducted in standard laboratories. In summary, HRM is a flexible method which can easily be adapted for the detection of other drug-resistance mediating mutations in the future. The next step of this ongoing study will be to screen a larger number of INH/RIF-R *M. tuberculosis* strains and to investigate the implementation of HRM method for detection of mutations involved in resistance to other first-line anti-TB drugs like ethambutol and streptomycin.

Acknowledgements

This work was supported by a grant from Tehran University of Medical Sciences (Project No. 20216).

References

1. Gillespie SH. Evolution of drug resistance in *Mycobacterium tuberculosis*: Clinical and molecular perspective. Antimicrob Agents Chemother 2002;46:267-74.
2. Riska PF, Jacobs WR Jr, Alland D. Molecular determinants of drug resistance in tuberculosis. Int J Tuberc Lung Dis 2000;4:S4-10.
3. Zhang Y, Yew WW. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis 2009;13:1320-30.
4. WHO. Global tuberculosis control. Geneva: World Health Organization; 2011.
5. Shamaei M, Marjani M, Chitsaz E, Kazempour M, Esmaeili M, Farnia P, *et al*. First-line anti-tuberculosis drug resistance patterns and trends at the national TB referral center in Iran-eight years of surveillance. Int J Infect Dis 2009;13:e236-40.
6. Reed GH, Kent JO, Wittwer CT. High-resolution DNA melting analysis for simple and efficient molecular diagnostics. Pharmacogenomics 2007;8:597-608.
7. Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, Murray MB. Tuberculosis drug resistance mutation database. PLoS Med 2009;6:e2.
8. Luo T, Zhao M, Li X, Xu P, Gui X, Pickerill S, *et al*. Selection of mutations to detect multidrug-resistant *Mycobacterium tuberculosis* strains in Shanghai, China. Antimicrob Agents Chemother 2010;54:1075-81.
9. Morgan M, Kalantri S, Flores L, Pai M. A commercial line probe assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: A systematic review and meta-analysis. BMC Infect Dis 2005;5:62.
10. Canetti G, Froman S, Grosset J, Hauduroy P, Langerova M, Mahler HT, *et al*. Mycobacteria: Laboratory methods for testing drug sensitivity and resistance. Bull World Health Organ 1963;29:565-78.
11. Van Soolingen D, Hermans PW, de Haas PE, Soll DR, van Embden JD. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: Evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol 1991;29:2578-86.
12. Ramirez MV, Cowart KC, Campbell PJ, Morlock GP, Sikes D, Winchell JM, *et al*. Rapid detection of multidrug-resistant *Mycobacterium tuberculosis* by use of real-time PCR and high-resolution melt analysis. J Clin Microbiol 2010;48:4003-9.
13. Zhang SL, Shen JG, Xu PH, Li DX, Sun ZQ, Li L, *et al*. A novel genotypic test for rapid detection of multidrug-resistant *Mycobacterium tuberculosis* isolates by a multiplex probe array. J Appl Microbiol 2007;103:1262-71.
14. Pietzka AT, Indra A, Stöger A, Zeinzinger J, Konrad M, Hasenberger P, *et al*. Rapid identification of multidrug-resistant *Mycobacterium tuberculosis* isolates by *rpoB* gene scanning using high-resolution melting curve PCR analysis. J Antimicrob Chemother 2009;63:1121-7.
15. Kapur V, Li LL, Iordanescu S, Hamrick MR, Wanger A, Kreisworth BN, *et al*. Characterization by automated DNA sequencing of mutations in the gene (*rpoB*) encoding the RNA polymerase b subunit in rifamipin resistant *Mycobacterium tuberculosis* strains from New York city and Texas. J Clin Microbiol 1994;32:1095-8.
16. Bang H, Park S, Hwang J, Jin H, Cho E, Kim DY, *et al*. Improved rapid molecular diagnosis of multidrug-resistant tuberculosis using a new reverse hybridization assay, REBA MTB-MDR. J Med Microbiol 2011;60:1447-54.
17. Ali A, Hasan Z, Moatter T, Tanveer M, Hasan R. *M. tuberculosis* Central Asian Strain 1 MDR isolates have more mutations in *rpoB* and *katG* genes compared with other genotypes. Scand J Infect Dis 2009;41:37-44.
18. Aslan G, Tezcan S, Serin MS, Emekdas G. Genotypic analysis of isoniazid and rifampin resistance in drug-resistant clinical *Mycobacterium tuberculosis* complex isolates in southern Turkey. Jpn J Infect Dis 2008;61:255-60.
19. Marttila HJ, Soini H, Eerola E, Vyshnevskaya E, Vyshnevskiy BI, Otten TF, *et al*. A Ser315Thr substitution

in *KatG* is predominant in genetically heterogeneous multidrug-resistant *Mycobacterium tuberculosis* isolates originating from the St. Petersburg area in Russia. Antimicrob Agents Chemother 1998;42:2443-5.
20. Gupta A, Prakash P, Singh SK, Anupurba S. Rapid genotypic detection of *rpoB* and *katG* gene mutations in *Mycobacterium tuberculosis* clinical isolates from Northern India as determined by MAS-PCR. J Clin Lab Anal 2013;27:31-7.
21. Hillemann D, Kubica T, Agzamova R, Venera B, Rüsch-Gerdes S, Niemann S. Rifampicin and isoniazid resistance mutations in *Mycobacterium tuberculosis* strains isolated from patients in Kazakhstan. Int J Tuberc Lung Dis 2005;9:1161-7.
22. Bakonyte D, Baranauskaite A, Cicenaite J, Sosnovskaja A, Stakenas P. Molecular characterization of isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates in Lithuania. Antimicrob Agents Chemother 2003;47:2009-11.

How to cite this article: Haeili M, Fooladi AI, Bostanabad SZ, Sarokhalil DD, Siavoshi F, Feizabadi MM. Rapid screening of *rpoB* and *katG* mutations in *Mycobacterium tuberculosis* isolates by high-resolution melting curve analysis. Indian J Med Microbiol 2014;32:398-403.

Source of Support: Tehran University of Medical Sciences. **Conflict of Interest:** None declared.