Frequency of mutations in rifampicin and isoniazid resistant isolates of *M. tuberculosis*: an analysis from Central India

Prabha Desikan[1,*], *Atul Kharate*[2], *Nikita Panwalkar*[3], *Jyoti Khurana*[4], *Shaina Beg Mirza*[5], *Aparna Chaturvedi*[6], *Reeta Varathe*[7], *Manju Chourey*[8], *Pradeep Kumar*[9], *Nitin Doshi*[10], *Manoj Pandey*[11]

Abstract

Background **The spread of drug-resistant tuberculosis has challenged tuberculosis control strategies globally. The present study aims to analyzes the frequency of mutations in *rpoB*, *katG* and *inhA* genes in strains of *M. tuberculosis* complex (MTBC) circulating in Central India. It is anticipated that the findings may provide a starting point to understand the evolutionary success of drug-resistant strains of MTBC in this region.**

Methods **Line probe assay was carried out on 720 consecutive sputum samples of MDR suspects from June 2012 to May 2013. Mutation frequencies in the *rpoB*, *katG* and *inhA* genes were analyzed.**

Results **Mutations were identified in 269 (37.6%) samples, as follows: 55 (7.6%) samples had mutations conferring resistance to only isoniazid, 84 (11.6%) had mutations conferring resistance to only rifampicin and 130 (18%) isolates had mutations conferring resistance to both isoniazid and rifampicin. The most frequent mutation in the *rpoB* gene was at codon S531L, seen in 141 (19.5%) isolates. The most frequent mutation in the *katG* gene was at codon S315T1, seen in 151 (20.9%) isolates; and in the *inhA* gene at codon C15T, seen in 21 (2.9%) isolates. Some unidentified mutations were also observed.**

Conclusion **The patterns and the frequency of the mutations identified in this study indicate the most frequent mutations at S531L codon in the *rpoB* gene, S315T1 codon in the *katG* gene and C15T codon in the promoter region of the *inhA* gene. Controlling the emergence and spread of MDR TB requires an understanding of the evolution of these mutations.**

Keywords **Mutation, frequency, Central India, *M. tuberculosis***

Background

The most recent report of the World Health Organization (WHO) on drug-resistant tuberculosis clearly indicates that the fight against tuberculosis is far from over.[1] The spread of drug-resistant tuberculosis has challenged tuberculosis control strategies globally.[2] The WHO-endorsed line probe assay (LPA), based on multiplex polymerase chain reactions, is used worldwide as a part of tuberculosis control programmes for simultaneous identification of

Received: 13 April 2016; revised: 26 July 2016; accepted: 23 August 2016

[1]MD, National Reference Laboratory, Department of Microbiology, Bhopal Memorial Hospital and Research Center, Bhopal, Madhya Pradesh, India; [2]MS, Intermediate Reference Laboratory, MRTB Hospital & Chest Center, Indore, Madhya Pradesh, India; [3]MSc, National Reference Laboratory, Bhopal Memorial Hospital and Research Center, Bhopal, Madhya Pradesh, India; [4]PhD, Intermediate Reference Laboratory, MRTB Hospital & Chest Center, Indore, Madhya Pradesh, India; [5]MSc, National Reference Laboratory, Bhopal Memorial Hospital and Research Center, Bhopal, Madhya Pradesh, India; [6]MSc, National Reference Laboratory, Bhopal Memorial Hospital and Research Center, Bhopal, Madhya Pradesh, India; [7]MSc, National Reference Laboratory, Bhopal Memorial Hospital and Research Center, Bhopal, Madhya Pradesh, India; [8]BSc, National Reference Laboratory, Bhopal Memorial Hospital and Research Center, Bhopal, Madhya Pradesh, India; [9]BSc, National Reference Laboratory, Bhopal Memorial Hospital and Research Center, Bhopal, Madhya Pradesh, India; [10]MSc, Intermediate Reference Laboratory, MRTB Hospital & Chest Center, Indore, Madhya Pradesh, India, [11]MS, Department of Oncology, Bhopal Memorial Hospital and Research Center, Bhopal, Madhya Pradesh, India.

*Corresponding author: Prabha Desikan, MD, National Reference Laboratory, Department of Microbiology, Bhopal Memorial Hospital and Research Center, Raisen Bypass Road, Karond, Bhopal, Madhya Pradesh, India. prabhadesikan@yahoo.com

Published December 2016

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

M. tuberculosis complex (MTBC) and detection of drug resistance to rifampicin (RIF) and isoniazid (INH) among these strains.[3-5] It gives results within 48 hours and provides information on the most common mutations and levels of resistance.[6]

We have attempted to analyze the frequency of mutations in the *rpoB*, *kat*G and *inh*A genes that code for RIF and INH sensitivity in strains of MTBC circulating in the state of Madhya Pradesh (India) by LPA. Madhya Pradesh, in Central India, is a densely populated region with 72.6 million people.[7] Currently, there is no data from this region on the prevalent mutation patterns of these genes.

Methods

Ethical approval was provided by the Institutional Ethics Committee (IEC) as per approval document IEC/15/Micro/14, dated 01.01.2015, of the Bhopal Memorial Hospital & Research Center (BMHRC), Bhopal. We analyzed the results generated from the LPA tests from the mandated diagnostic workflow of the two accredited Mycobacteriology laboratories in the state of Madhya Pradesh in Central India (National Reference Laboratory, BMHRC, Bhopal and Intermediate Reference Laboratory, Indore). LPA was carried out on sputum samples of MDR suspects defined as per criteria 'C' of the Revised National Tuberculosis Control Programme (RNTCP) (MDR suspect criteria detailed in Table 1). Samples were received from all districts of Madhya Pradesh under the RNTCP from June 2012 to May 2013. A total of 720 sputum samples were processed within 48 hours of collection by the NALC-NaOH decontamination method.[8] The sediments were suspended in 1 mL sterile phosphate buffer (pH 6.8). Smears of the sediments were stained Ziehl-Neelsen. DNA extraction of all smear-positive decontaminated samples was carried out using the GenoLyse® kit (Hain Lifescience GmbH, Nehren, Germany) as per manufacturer's instructions.[9] The GenoType MTBDR*plus* (Hain Lifescience GmbH, Nehren, Germany) line probe assay for detection of rifampicin and/or isoniazid resistance was carried out according to the manufacturer's instructions.[10]

Table 1. MDR suspect criteria as defined by the Revised National Tuberculosis Control Programme, India

Criteria A • All failures of new TB cases • Smear positive previously treated cases who remain smear positive from the 4th month onwards • All pulmonary TB cases who are contacts of known MDR TB cases
Criteria B – *in addition to Criteria A:* • All smear positive previously treated pulmonary TB cases at diagnosis • Any smear positive follow up result in new or previously treated cases
Criteria C – *in addition to Criteria B* • All smear negative previously treated pulmonary TB cases at diagnosis, • HIV TB coinfected cases at diagnosis

Mutations in codons 505 to 533 of the *rpoB* gene were analyzed. The four mutation probes for the *rpoB* gene on the LPA strip detected the mutations at the D516V (*rpoB* MUT1), H526Y (*rpoB* MUT2A), H526D (*rpoB* MUT2B) and S531L (*rpoB* MUT3). The two mutation probes for the *kat*G gene detected mutations at the codons S315T1 (*kat*G MUT1) and S315T2 (*kat*G MUT2). The four mutations probes for the *inh*A gene detected mutations at the codons C15T (*inh*A MUT1), A16G (*inh*A MUT2), T8C (*inh*A MUT3A) and T8A (*inh*A MUT3B). Missing wild type bands represented known/unknown mutation/s.

On an assay strip, 21 reaction zones representing various wild types (WT) and mutation (MUT) bands of the *rpoB*, *kat*G and *inh*A genes were probed. The presence of mutation band/s at *rpoB*, *kat*G or *Inh*A gene regions or lack of at least one of the wild-type bands was considered indicative of resistance to the respective drug. The presence of all wild-type bands and absence of mutation bands was indicative of susceptibility to the drug. Each individual test was verified for the presence of five control zones that included a conjugate control, an amplification control, and three locus control zones (*rpoB*, *kat*G and *inh*A). The

assay was considered valid if bands for master mix and extraction negative controls were absent, and bands for conjugate and amplification controls were present.

Results

Out of a total of 720 DNA isolates from 720 samples, mutations were identified in 269 (37.6%) isolates. Fifty five (7.6%) isolates had mutations conferring resistance to only INH (INH monoresistant), 84 (11.6%) had mutations conferring resistance to only RIF (RIF monoresistant) and 130 (18%) isolates had mutations conferring resistance to both INH and RIF (multidrug resistant or MDR isolates).

Mutations in the *rpoB* gene

There were 29 different band patterns detected in the *rpoB* gene, which codes for the β subunit of RNA polymerase and sensitivity to RIF. The most frequent mutation was detected at codon S531L, which was seen in 141 (19.5%) isolates. The second most prevalent mutation in the *rpoB* gene was at codon D516V, which was seen in 21 (2.9%) isolates. The frequency of mutations at codons H526Y and H526D were found to be 1.3% (10 isolates) and 0.9% (7 isolates) respectively.

Forty three (5.9%) isolates had one or more missing WT bands while none of them were accompanied by MUT band in the hot spot region (81 base pair region), which is the rifampicin resistance-determining region of the *rpoB* gene, indicating unknown mutations. There were 23 isolates (3.1%) in which a single WT band deletion was found. Single WT2 band deletion was seen in 12 (1.6%) isolates, single WT3 band deletion in 3 (0.4%) isolates, single WT4 band deletion in 1 (0.1%) isolate, single WT7 band deletion in 5 (0.6%) isolates and single WT8 band deletion in 2 (0.2%) isolates. Two simultaneous WT band deletions with absence of MUT band were found in 19 (2.6%) isolates. WT 1 and 7 deletion in 2 (0.2%) isolates, WT 2 and 3 deletion in 3 (0.4%) isolates, WT 2 and 7 deletion in 1 (0.1%) isolate, WT 3 and 4 deletion in 9 (1.2%) isolates, WT 4 and 5 deletion in 2 (0.2%) isolates, WT 5 and 6 deletion and WT 7 and 8 deletion in 1 (0.1%) isolate each were seen. There was only 1 (0.1%) isolate in which 3 WT bands were found to be deleted simultaneously along with absence of MUT band. This isolate showed deletion at 2, 3 and 4 WT band positions.

All WT bands (1 to 8) of the *rpoB* gene were present in 596 (82.7%) isolates. Five hundred and six (70.2%) isolates did not show any mutant DNA sequences, thus indicating sensitivity to RIF. Ninety (12.5%) isolates displayed varied mutations along with presence of all WT bands. There were 70 (9.7%) isolates with MUT 3 band representing mutation at codon S531L, 6 (0.8%) isolates with MUT1 band indicating mutation at codon D516V, 5 (0.6%) isolates with MUT2A band indicating mutation at codon H526Y, 3 (0.41%) isolates with MUT2B representing mutation at codon H526D. Four (0.5%) isolates having have both MUT1 and MUT3 bands representing mutations at codons D516V and S531L respectively; 1 (0.1%) isolate with presence of both MUT2A and MUT3 bands representing mutations at codons H526Y and S531L and 1 (0.1%) isolate with presence of all 4 mutation bands; MUT1, MUT2A, MUT2B and MUT3, representing mutations at codons D516V, H526Y, H526D and S531L respectively were seen.

Eighty one isolates (11.2%) had one or more missing WT bands accompanied by a mutation band. There were 61 isolates (8.4%) with absence of WT8 band and presence of MUT3 band (indicating mutation at codon S531L); 10 (1.3%) isolates with absence of both WT3 and WT4 bands and presence of MUT1 band (indicating mutation at codon D516V); 3 (0.4%) isolates with absence of both WT2 and WT8 bands along with presence of MUT3 probe (indicating mutation at codon S531L); 3 (0.4%) isolates with absence of WT7 along with the presence of MUT2B (indicating mutation at codon H526D) band; 2 (0.2%) isolates with missing WT7 band and presence of MUT2A band (indicating mutation at codon H526Y); 1 (0.1%) isolate with absence of both WT2 and WT8 band accompanied by presence of MUT2A band (indicating mutation at codon H526Y) and 1 (0.1%) isolate with absence of both WT3 and

Table 2. Banding patterns obtained by line probe assay in the *rpoB*, *katG* and *inhA* genes

Gene	Δ WT	WT	Mutation	No (%)
rpoB	Δ WT2,8	WT1,3,4,5,6,7	S531L	3 (0.4%)
	Δ WT2,8	WT1,3,4,5,6,7	H526Y	1 (0.1%)
	Δ WT3,4	WT1,2,5,6,7,8	D516V	10 (1.3%)
	Δ WT3,4	WT1,2,5,6,7,8	S531L	1 (0.1%)
	Δ WT7	WT1,2,3,4,5,6,8	H526D	3 (0.4%)
	Δ WT7	WT1,2,3,4,5,6,8	H526Y	2 (0.2%)
	Δ WT8	WT1,2,3,4,5,6,7	S531L	61 (8.4%)
	...	WT1,2,3,4,5,6,7,8	D516V	6 (0.8%)
	...	WT1,2,3,4,5,6,7,8	H526Y	5 (0.6%)
	...	WT1,2,3,4,5,6,7,8	H526D	3 (0.4%)
	...	WT1,2,3,4,5,6,7,8	S531L	70 (9.7%)
	...	WT1,2,3,4,5,6,7,8	D516V & S531L	4 (0.5%)
	...	WT1,2,3,4,5,6,7,8	H526Y & S531L	1 (0.1%)
	...	WT1,2,3,4,5,6,7,8	D516V, H526Y, H526D & S531L	1 (0.1%)
	Δ WT2	WT1,3,4,5,6,7,8	...	12 (1.6%)
	Δ WT3	WT1,2,4,5,6,7,8	...	3 (0.4%)
	Δ WT4	WT1,2,3,5,6,7,8	...	1 (0.1%)
	Δ WT7	WT1,2,3,4,5,6,8	...	5 (0.6%)
	Δ WT8	WT1,2,3,4,5,6,7	...	2 (0.2%)
	Δ WT1,7	WT2,3,4,5,6,8	...	2 (0.2%)
	Δ WT2,3	WT1,4,5,6,7,8	...	3 (0.4%)
	Δ WT2,7	WT1,3,4,5,6,8	...	1 (0.1%)
	Δ WT3,4	WT1,2,5,6,7,8	...	9 (1.2%)
	Δ WT4,5	WT1,2,3,6,7,8	...	2 (0.2%)
	Δ W5,6	WT1,2,3,4,7,8	...	1 (0.1%)
	Δ WT7,8	WT1,2,3,4,5,6	...	1 (0.1%)
	Δ WT1,2,3	WT4,5,6,7,8	...	1 (0.1%)
	...	WT1,2,3,4,5,6,7,8	...	506 (70.2%)
katG	Δ WT	...	S315T1	142 (19.7%)
	Δ WT	...	...	18 (2.5%)
	...	WT	S315T1	8 (1.1%)
	...	WT	S315T1 & S315T2	1 (0.1%)
	...	WT	...	551 (76.5%)
inhA	Δ WT1	WT2	C15T	18 (2.5%)
	Δ WT1,2	...	...	1 (0.1%)
	Δ WT1	WT2	...	1 (0.1%)
	...	WT1,2	C15T	3 (0.4%)
	...	WT1,2	T8C	3 (0.4%)
	...	WT1,2	...	694 (96.3%)

WT – wild type, **Δ WT** – absence of wild type band.

WT4 with presence of MUT3 band (indicating mutation at codon S531L) – Table 2.

Mutations in the *katG* gene

There were six different band patterns detected in the *kat*G gene, which codes for catalase peroxidase enzyme and sensitivity to INH. Five hundred and fifty one (76.5%) isolates were sensitive to INH, and showed a WT band without any MUT band. Mutation in the *kat*G gene at the codon S315T1 was found to be the most frequent, with 151 (20.9%) isolates showing this mutation. Nine (1.2%) isolates displayed at least one MUT band along with presence of the WT band. Eight (1.1%) isolates had MUT1 band indicating mutation at codon S315T1 along with presence of the WT band, and a single (0.1%) isolate had both MUT1 and MUT2 bands indicating mutations at codon S315T1 and S315T2 respectively along with presence of the WT band.

There were 142 (19.7%) isolates that did not have the WT band, and had only MUT1 band (indicating mutation at codon S315T1). The entire *kat*G gene was absent in 18 (2.5%) isolates. There were a total of 151 (20.9%) isolates with MUT 1 band (with or without the WT band), indicating mutation at codon S315T1 (Table 2).

Mutation in the *inhA* gene

Six different band patterns were identified at the promoter region of *inh*A gene, which codes for the NADH enoyl ACP reductase enzyme and sensitivity to INH. There were 21 isolates (2.9%) with MUT1 band, indicating mutation at codon C15T and 3 isolates (0.4%) with MUT3A band, indicating mutation at codon T8C. Six hundred ninety four isolates (96.3%) with presence of all WT bands did not show any MUT band representing sensitivity to INH. Six (0.8%) isolates had all WT and at least one MUT band in the *inh*A gene. Three (0.4%) isolates had MUT1 band indicating mutation at codon C15T, along with presence of all WT bands and 3 (0.4%) had MUT3A band indicating mutation at T8C, along with simultaneous presence of both WT1 and WT2 bands. Eighteen (2.5%) isolates had only a WT2 band and a MUT1 band. The entire *inh*A gene was deleted in 1 (0.1%) isolate. Unknown mutation was represented by the absence of WT1 band accompanied by the presence of WT2 and MUT1 band (indicative of mutation at codon C15T) and was found in 1 (0.1%) isolate (Table 2).

Discussion

M. tuberculosis exhibits multiple mechanisms to inactivate anti-TB drugs owing to point mutations, insertions or deletions in the genes coding for sensitivity to the respective drug.[11] LPA detects common mutations in the *rpoB*, *kat*G and *inh*A genes that result in resistance to RIF and INH. Earlier studies have shown that the frequency of mutation is highest (96%) in the core, hot spot region of the rifampicin resistance-determining region of the *rpoB* gene.[12-14] Van Deun A et al.[15] have reported 46.9% and 63%, S531L mutation frequencies from Bangladesh and Kinshasa respectively. These studies indicate that the most frequent mutation occurs at codon 531 of the *rpoB* gene that results in amino acid exchange from serine to leucine. Our data are consistent with the above findings, exhibiting the most frequent mutation in S531L codon in the *rpoB* gene. We also found mutations at codons D516V, H526D and H526Y most frequently. Van Deun A et al.[15] however, have reported D516V mutation frequencies of 3.4% and 7.1%; H526D mutation frequencies of 6.9% and 2%; and H526Y mutation frequencies of 9.7% and 2.4% from Bangladesh and Kinshasa respectively. It appears that these mutation frequencies vary widely across geographical areas.

The predominant band patterns in RIF-resistant strains in our study were absence of WT3 and WT4 accompanied by the presence of MUT 1 probe; deletion of WT 7 band accompanied by the presence of MUT2A or MUT2B band and presence of MUT 3 band with absence of WT 8 band. This pattern of absence of specific WT band/s accompanied by presence of specific MUT band/s was consistent with the description in the laboratory manual for LPA.[16] However, two unusual patterns were observed in this study. One was absence of WT2 and WT8 bands with presence of MUT 2A band (instead of MUT3 band), and the other was absence of WT3 and WT4 bands with presence of MUT3 probe band (instead of MUT2A or MUT2B band). These unique patterns may indicate that there are uncommon mutations in strains of MTB circulating in this geographical area. This emphasizes the need for phenotypic confirmation of atypical LPA results, that is, by culture and drug sensitivity testing.[13] The presence of WT along with MUT bands in RIF-resistant isolates expressed either heteroresistance or mixed infection. The assay could not differentiate between the two.

Mutation of the S315T codon was the most common mutation of the *kat*G gene in our study. This is similar to results from earlier studies.[13,17-19] An unusual pattern was observed in one isolate in which WT deletion band was not accompanied by a concomitant presence of a MUT band. This may be due to mutation of one

or more genes other than *kat*G and *inh*A, notably *ahp*C, *oxy*R, *kas*A, *fur*A and *ndh*.[20,21]

There were 6.11% unknown mutations in our study. These mutations may or may not have been present in this region earlier. Van Deun A et al.[15] classified 511Pro, 516Tyr, 526Asn, 526Leu, 526Ser, 533Pro, and 572Phe *rpoB* mutations as "disputed" resistance, based on the discordant RIF drug susceptibility testing results. The unknown mutation patterns in the *rpoB* gene in our study may belong to the "disputed" resistance patterns as described by them. Further studies, using DNA sequencing, are needed to identify the nature of these mutations.

A limitation of the present study is that clinical data was not available for the patients.

Conclusion

In the *rpoB* gene, the frequency of mutation was the highest at the S531L codon in the hot spot core region. Mutation frequency was the highest at the S315T1 codon in the *kat*G gene, and at the C15T codon in the promoter region of the *inh*A gene. Varied banding patterns were recognized by LPA indicating heteroresistance in the population. A considerable number of unidentified mutations were also observed. Further studies to elucidate these mutations would enhance our understanding of the epidemiology and transmission dynamics of drug resistant-strains in this geographical area and help provide the basis for effective strategies for control of drug-resistant TB in this region.

Authors' contributions statement: PD conceptualized and wrote the manuscript, analyzed and interpreted the data. AK helped write the manuscript, analyzed and interpreted the data. NP helped write the manuscript, analyzed and interpreted data. JK analyzed and interpreted the data. SBM, AC, RV, MC, PK, ND: carried out bench work, generated data. MP critically revised and approved the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: The authors acknowledge funding support from the Foundation for Innovative New Diagnostics (FIND), through the Revised National Tuberculosis Control Programme of India.

References

1. World Health Organization. Global Tuberculosis Report 2014. Geneva, Switzerland 2014. Accessed on: 03 Mar 2016. Available at: http://www.aidsdatahub.org/global-tuberculosis-report-2014-who-2014.
2. Sharma SK, Mohan A. Tuberculosis: From an incurable scourge to a curable disease - journey over a millennium. Indian J Med Res 2013;137:455-93. [PubMed] [FullText]
3. Molecular line probe assays for rapid screening of patients at risk of multi-drug resistant tuberculosis (MDR-TB). Policy Statement. Geneva, Switzerland: World Health Organization; 2008. Accessed on: 03 Mar 2016. Available at: http://www.who.int/tb/features_archive/policy_statement.pdf.
4. Anti-tuberculosis drug resistance in the world. Fourth global report. The WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance 2002-2007. Geneva, Switzerland: World Health Organization; 2008. Accessed on: 03 Mar 2016. Available at: http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf.
5. Nikolayevskyy V, Balabanova Y, Simak T, et al. Performance of the Genotype MTBDRPlus assay in the diagnosis of tuberculosis and drug resistance in Samara, Russian Federation. BMC Clin Pathol 2009;9:2. [Crossref] [PubMed] [FullText]
6. Raveendran R, Wattal C, Oberoi JK, Goel N, Datta S, Prasad KJ. Utility of GenoType MTBDRplus assay in rapid diagnosis of multidrug resistant tuberculosis at a tertiary care centre in India. Indian J Med Microbiol 2012;30:58-63. [Crossref] [PubMed]
7. Provisional Population Totals: Census Info India, 2011. Accessed on: 03 Mar 2016. Available at: http://censusindia.gov.in/2011census/censusinfodashboard/
8. Revised National TB Control Programme, Training manual for *Mycobacterium tuberculosis* culture & drug susceptibility testing, Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan.
9. GenoLyse® Hain VER 1.0, Instructions for Use, IFU-51610-09, Hain Life science. 2/2012. Accessed on: 03 Mar 2016. Available at: http://www.ipaqt.org/wp-content/uploads/2013/02/GenoLyse_Istructions-for-use.pdf
10. GenoType MTBDR plus, VER 2.0, Instructions for Use, IFU-304A-02, Hain Life sciences. 2/2012. Accessed on: 03 Mar 2016. Available at: http://www.ipaqt.org/wp-content/uploads/2013/02/MTBDRplusV2_product-insert.pdf
11. Siddiqi N, Shamim M, Hussain S, et al. Molecular characterization of multidrug-resistant isolates of *Mycobacterium tuberculosis* from patients in North India. Antimicrob Agents Chemother 2002;46:2443-2450. [Crossref] [PubMed] [FullText]
12. Bártfai Z, Somoskövi A, Ködmön C, et al. Molecular characterization of rifampin-resistant isolates of *Mycobacterium tuberculosis* from Hungary by DNA sequencing

and the line probe assay. J Clin Microbiol 2001;39:3736-9. [Crossref] [PubMed] [FullText]
13. Bang D, Bengård Andersen A, Thomsen VØ. Rapid genotypic detection of rifampin- and isoniazid-resistant *Mycobacterium tuberculosis* directly in clinical specimens. J Clin Microbiol 2006;44:2605-8. [Crossref] [PubMed] [FullText]
14. Vijdea R, Stegger M, Sosnovskaja A, Andersen AB, Thomsen VØ, Bang D. Multidrug-resistant tuberculosis: rapid detection of resistance to rifampin and high or low levels of isoniazid in clinical specimens and isolates. Eur J Clin Microbiol Infect Dis 2008;27:1079-86. [Crossref] [PubMed]
15. Van Deun AV, Aung KJ, Bola V, et al. Rifampin drug resistance tests for tuberculosis: Challenging the gold standard. J Clin Microbiol 2013;51:2633-40. [Crossref] [PubMed] [FullText]
16. Barnard M, Parsons L, Miotto P, et al. Molecular detection of drug resistant tuberculosis by line probe assay. Laboratory manual for resource limited settings. FIND 2012.
17. Mokrousov I, Narvskaya O, Otten T, Limeschenko E, Steklova L, Vyshnevskiy B. High prevalence of *kat*G Ser315Thr substitution among isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates from northwestern Russia, 1996 to 2001. Antimicrob Agents Chemother 2002;46:1417-24. [Crossref] [PubMed] [FullText]
18. Musser JM, Kapur V, Williams DL, Kreiswirth BN, van Soolingen D, van Embden JD. Characterization of the catalase-peroxidase gene (*kat*G) and *inh*A locus in isoniazid-resistant and -susceptible strains of *Mycobacterium tuberculosis* by automated DNA sequencing: restricted array of mutations associated with drug resistance. J Infect Dis 1996;173:196-202. [Crossref] [PubMed]
19. Hillemann D, Weizenegger M, Kubica T, Richter E, Niemann S. Use of the genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* complex isolates. J Clin Microbiol 2005;43:3699-703. [Crossref] [PubMed] [FullText]
20. Coovadia YM, Mahomed S, Pillay M, Werner L, Mlisana K. Rifampicin mono-resistance in *Mycobacterium tuberculosis* in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region. PLoS ONE 2013;8:e77712. [Crossref] [PubMed] [FullText]
21. Telenti A, Honore N, Bernasc C, et al. Genotypic assessment of isoniazid and rifampin resistance in *Mycobacterium tuberculosis*: a blind study at reference laboratory level. J Clin Microbiol 1997;35:719-23. [PubMed] [FullText]

Please cite this article as:
Desikan P, Kharate A, Panwalkar N, Khurana J, Mirza SB, Chaturvedi A, Varathe R, Chourey M, Kumar P, Doshi N, Pandey M. Frequency of mutations in rifampicin and isoniazid resistant isolates of M. *tuberculosis*: an analysis from Central India. GERMS 2016;6(4):125-131. doi: 10.11599/germs.2016.1096