

Pattern of *InhA* and *KatG* mutations in isoniazid mono-resistant *Mycobacterium tuberculosis* isolates

Ashok Singh Charan¹, Neeraj Gupta¹, Ramakant Dixit¹, Piyush Arora¹, Tarun Patni², Kalliath Antony¹, Manisha Singh³

¹Department of Respiratory Medicine, JLN Medical College, Ajmer, Rajasthan, India, ²State TB Demonstration Centre, Ajmer, Rajasthan, India, ³Department of Psychiatry, JLN Medical College, Ajmer, Rajasthan, India

ABSTRACT

Aims and Objectives: The aim of the study is to detect the pattern of genetic mutation, i.e., *InhA* or *KatG* or both (*InhA* and *KatG*) in isoniazid (INH) mono-resistant mycobacteria and to correlate with the pattern in multidrug-resistant (MDR) isolates. **Materials and Methods:** In this study, a quantitative research approach was used. The research design was descriptive observational study. The study was conducted at the Department of Respiratory Medicine, JLN Medical College, Ajmer, Rajasthan, and Intermediate Referral Laboratory, State TB Demonstration Centre, Ajmer. A total of 298 samples found to have resistant strains of *Mycobacterium tuberculosis* were enrolled with purposive sampling. **Results:** The mean age of patients was 40.27 ± 13.82 years. There were 250 (83.9%) males, while 48 (16.1%) were females. One hundred ninety-two (64.4%) were resistant for INH only, while the rest were resistant to both INH as well as rifampicin (MDR-tuberculosis). The most common mutation in INH mono-resistance was *katG* (125; 65.1%) as compared to *inhA* (54; 28.1%) and both *inhA* and *katG* (13; 6.7%). Among *katG* mutations, the most common gene pattern was the absence of WT (S315T) and the presence of MUT1 (S315T1). **Conclusion:** Knowledge about mutation patterns of different INH resistant strains is important in the present era where there is a provision of separate regimens for INH mono-resistant TB. Since these mutations are very closely related to high- or low-degree resistance to INH, the therapeutic regimens cannot be generalized.

KEY WORDS: Gene pattern, *InhA*, isoniazid resistance, *KatG*, multidrug-resistant tuberculosis, mutations

Address for correspondence: Dr. Neeraj Gupta, 467, Behind Dayal Veena Trust, Civil Lines, Ajmer - 305 001, Rajasthan, India.
E-mail: drneerajajmer@yahoo.com

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INTRODUCTION

Drug resistance in tuberculosis (TB) is a major public health challenge in developing countries, and the emergence of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains has become a major obstacle in the management of TB.^[1] Isoniazid (INH) is one of the most potent antimycobacterial agents available for the treatment of TB and has both bactericidal and sterilizing actions.

It inhibits mycolic acid biosynthesis of *M. tuberculosis*. However, the resistance to INH is most common among all first-line anti-TB drugs.^[2] As far as mono-resistance is concerned, resistance to INH (7.2%) exceeds other first-line anti-TB drugs (6.85% for streptomycin, 1.6% for ethambutol, and 4.6% for rifampicin).^[3]

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The results from studies conducted at Chennai and Bangalore have found an MDR-TB incidence of 12% in re-treatment cases and around 1%–3% in new cases.^[4] According to the global annual surveys conducted by the World Health Organization (WHO), an estimated 490,000 cases of MDR-TB occurred among 10.4 million new active TB cases in 2016.^[5] Three countries accounted for almost half of the world's cases of MDR/RR-TB: India (24%), China (13%), and the Russian Federation (10%). Globally, 3.5% of new TB cases and 18% of previously treated cases had MDR/rifampicin-resistant TB.^[6]

The genetic background of *M. tuberculosis* related to INH resistance is very complex. Mutations in several genes, including *KatG* (catalase-peroxidase coding genes), *InhA*, *AhpC*, and *KasA*, have all been associated with INH resistance. Two molecular mechanisms have been shown to be the main cause of INH resistance, i.e., mutations in *KatG* and *InhA*. From 50% to 95% of INH-resistant strains contain mutations in codon 315 (WT1) of the *KatG* gene. Furthermore, 20%–35% of INH-resistant strains contain mutations in the *InhA* regulatory region.^[7,8] The *inhA* gene encodes an enoyl-acyl carrier protein reductase involved in the fatty acid synthesis.^[9]

The recognition of INH-resistance patterns and the frequency of *katG* and *inhA* mutations in different geographic areas may help to guide decision-making about standardization of treatment regimens or individualized treatment, as in the case of INH monoresistance or in the case of MDR-TB or extensively drug-resistant (XDR)-TB.^[10] As mutations in *katG*, particularly at codon 315, confer high-level INH resistance, even high-dose INH is ineffective for the treatment of *M. tuberculosis* with this mutation profile. Mutations in *inhA*, on the other hand, may respond to high doses of INH.^[11]

There is no documented study on molecular characterization of INH resistance with reference to monoresistance in the Western part of India. Majority of studies are from out of the country and very few studies are reported from India. Hence, this was of interest to analyze the *KatG* gene and *InhA* mutations in INH monoresistant isolates in this part of the country and to compare the pattern with other national and internationally available data.

MATERIALS AND METHODS

In this study, a quantitative research approach was adopted with a descriptive observational study. The study was conducted at Intermediate Referral Laboratory, Ajmer, and Respiratory Medicine Department, JLN Medical College, Ajmer, Rajasthan. Permission to use the data was obtained from the Intermediate Reference Laboratory, Ajmer, and approval was obtained from the institutional ethical committee, JLN Medical College, Ajmer. A total of 298 samples reported to be resistant

to INH with or without RMP were enrolled through purposive sampling. The study duration was from August 1, 2017, to October 30, 2018.

The method used to detect the INH resistance was genotype MDR TB plus, version 2, Hain Lifesciences Nehren, Germany (first-line Line Probe Assay), made available under the Revised National Tuberculosis Control Program. The nucleotide sequences of the *katG* and *inhA* gene were recorded for each of the samples.

Data analysis was done with the use of statistical software SPSS 17.0 (IBM SPSS Version 17.0, Chicago).

RESULTS

A total of 298 consecutive samples found to have either INH monoresistant or MDR strains were included in the study and were analyzed.

Figure 1 depicts that around two-third of the study population, that is, 192 (64.4%) were resistant for INH, while the rest were 106 (35.6) resistant to both INH and rifampicin (MDR-TB).

The mean age of the patients was 40.27 ± 13.82 years. There were 250 males (83.9%), while only 48 (16.1%) were females. Table 1 shows that 163 (65.2%) of the males and 29 (60.4%) of the females were resistant to INH alone, while the rest were resistant to both INH and RMP. No significant difference was observed for INH resistance or MDR-TB between the two genders. Among INH-resistant patients, 73 (38.0%) were between the age group of 31–45 years and 44 (22.9%) were between the age group of 15–30 years, while in MDR patients, 41 (38.7%) were between the age group of 15–30 years. This difference was found to be statistically significant ($P = 0.03$).

The most common mutation in INH resistant strains was in *katG* gene (125, 65.1%) followed by *inhA* gene (54, 28.1%).

Table 1: Association of type of resistance to INH or H&R with demographic variables of patients (n=298)

Demographic Variables	H Monoresistant		H & R Resistant (MDR)		P
	n	%	n	%	
Sex					
Male	163	65.2	87	34.8	0.51NS
Female	29	60.4	19	49.6	
Age (in years)					
15-30	44	22.9	41	38.7	0.03*
31-45	73	38.0	35	33.0	
46-60	61	31.8	25	23.6	
>60	14	7.3	5	4.7	

NB: * = Significant at $P < 0.05$, NS = Non-significant $P > 0.05$

Table 2: Pattern of gene mutations in INH Monoresistant mycobacterium TB strains (n=196)

<i>inhA</i> n (%)	<i>katG</i> n (%)	Both n (%)
58 (28.1%)	125 (65.1%)	13 (6.7%)

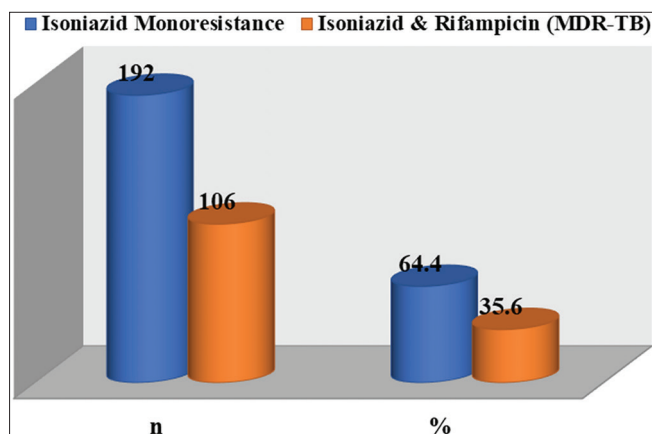


Figure 1: Total number of isolates registered for the study

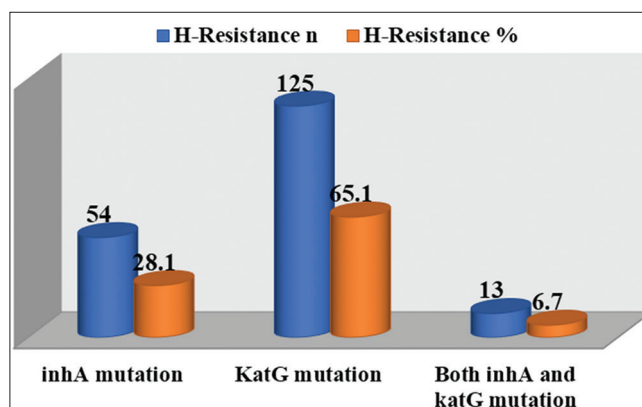


Figure 2: Pattern of genes in Isoniazid(H) Mono-resistance Tuberculosis Patients (N=192)

Table 3: Analysis of patterns of gene mutations of *InhA* and *katG* in Isoniazide (H) Mono-resistant Tuberculosis Patients (n=179)

Pattern of Gene Mutations	Locus											
	WT1(-)		WT2 (-)		MUT1 (+)		MUT2 (+)		MUT3A(+)		MUT3B(+)	
	n	%	n	%	n	%	n	%	n	%	n	%
<i>InhA</i> Gene (58)	48	82.7	9	15.5	37	63.8	0	0.0	5	8.6	1	1.7
<i>katG</i> Gene (125)	122	97.6	0	0	117	93.6	2	1.6	0	0	0	0

In 13 (6.7%) patients, both *inhA* and *katG* gene mutations were observed [Figure 2 and Table 2].

Comparing the pattern of gene mutations in INH mono-resistant strains revealed that of 54 isolates with only *inhA* gene mutation, WT1 pattern was absent in 48 (88.9%) strains, while MUT1 pattern was present in 37 (68.5%) strains. *KatG* gene mutation patterns were observed in different sequences. WT was absent in 97.6% of patients, MUT1 was seen in 117 (93.6%) patients, while in only 2 (1.6%) of patients, MUT2 resistance was observed [Table 3].

The occurrence of mutation at various loci of *katG* and *inhA* genes was also very similar for MDR TB isolates as that of INH mono-resistant strains [Table 4].

The overall incidence of occurrence of *inhA*, *katG*, and combined pattern of mutation did not differ in MDR TB [Table 5], and *katG* was the most common mutation observed for both the groups.

DISCUSSION

In the present study, of 298 patients, the mean age of the study population was 40.27 ± 13.82 years. One hundred ninety-three (64.7%) of 298 patients were in the age group of 15–45 years. Eighty-six (28.2%) patients followed by 19 (6.4%) patients were in the age group 45–60 years and more than 60 years, respectively. The mean age reported in other studies was 28.43 ± 14.32 years^[12] and 26 years.^[13] Thus, all these studies revealed similar age distribution among drug-resistant patients and clearly suggest that

Table 4: Occurrence of mutations in *inhA* and *katG* genes in MDR TB Strains

<i>inhA</i>	n=5
WT1(-)	5 (100%)
WT2(-)	1 (20%)
MUT1(+)	4 (80%)
MUT2(+)	0 (0%)
MUT3A(+)	1 (20%)
MUT3B(+)	0 (0%)
<i>katG</i>	n=96
WT(-)	90 (93.8%)
MUT1(+)	85 (88.5%)
MUT2(+)	1 (1%)

Table 5: Line probe assay based band pattern analysis of all drug-resistant (H & HR) *Mycobacterium tuberculosis* strains (n=298)

INH mono-resistant n=192	MDR TB n=106	Total n=298	Percentage
<i>inhA</i> 54 (28.1%)	<i>inhA</i> 5 (4.7%)	59	19.7
<i>katG</i> 125 (65.1%)	<i>katG</i> 96 (90.5%)	221	74.1
Both 13 (6.7%)	Both 5 (4.7%)	18	6.0

drug-resistant TB affects younger and economically productive age groups.

In the present study, most of the patients were male 250 (83.9%), while 48 (16.1%) were female. The male-to-female ratio was 5:1. Similar gender distribution was reported by Vilegas *et al.* (2016), i.e., 82.1% were male and 17.9% were female.^[14] Surkova *et al.*, Belarus, also reported similar gender distribution (934 patients; 660 were males [$70.67 \pm 1.5\%$] and 274 were females [$29.33 \pm 1.5\%$]) ($P < 0.001$).^[15]

Table 6: Comparative results of mutation patterns reported from different parts of world

Study/authors	Country	Number of patients (n)	<i>InhA</i> mutation	<i>KatG</i> mutation	Both
C. Alagappan <i>et al.</i> (2018)	India	1821	528 (29)	1297 (71)	-
J. Isakova <i>et al.</i> (2018)	Russia	114	8 (7)	104 (91.2)	-
Z. Tavakkoli, A. Nazem <i>et al.</i> (2018)	Brazil	116	20 (17.24)	96 (82.6)	-
V. R. Bollela <i>et al.</i> (2016)	Brazil	22	9 (40.9)	12 (54.5)	1 (4.5)
	Mozambique	38	4 (10.5)	32 (84.2)	2 (4.2)
Abraham J. Nieshaus <i>et al.</i> (2015)	South Africa	603	99 (16.41)	435 (72.13)	69 (11.44)
T. Jagielski <i>et al.</i> (2015)	Poland	54	2 (3.7)	46 (88.2)	6 (11.1)
N. Shubludze <i>et al.</i> (2013)	Georgia	678	143 (22.6)	535 (84.3)	-
T. Luo <i>et al.</i> (2009)	China	242	24 (9.9)	216 (89.3)	02 (0.8)
Elis R Dalla Costa <i>et al.</i> (2009)	South America	224	43 (20)	181 (80)	-
Present study (2019)	India	298	59 (19.7)	221 (74.1)	18 (6)

Patterns observed in the present study are almost similar to that reported by others

Of 298 drug-resistant isolates, 191 (64.4%) were INH monoresistant, while 107 (35.6%) isolates were resistant to both INH and rifampicin. The INH monoresistance group comprised 163 (84.89%) males and 29 (15.1%) females, while in Multi drug resistance patients, there were 87 (82.07%) males and 19 (17.2%) females.

In the present study, we observed that the most common mutation in INH monoresistant patients was in *katG* gene (125, 65.1%), followed by *inhA* gene which was observed in 54 (28.1%) patients, whereas in 13 (6.7%) patients, both *inhA* and *katG* gene mutations were observed. Almost similar results were reported by Yao *et al.* They observed that of 50 INH monoresistant patients, 41 (82%) had *KatG* mutations and 9 (18%) had *inhA* mutations.^[16] Huyen *et al.* also reported that 75.3% had mutations in *katG* and 28.2% had mutations in the *inhA* promoter region.^[17] A study by Kigozi *et al.* similarly observed that 80% had *katG* mutations and 6% had *inhA* mutations.^[18] Tavakkoli and Nazemi also reported that in 17.24% and 82.76% of the strains, *inhA* genes and *katG* genes, respectively, were responsible for INH resistance.^[19] Niehaus *et al.* found that 33.1% of 924 isolates had an *inhA* mutation with or without a *katG* mutation, representing 30.3% of those with MDR-TB, 47.2% of those with pre-XDR-TB, and 82.8% of those with XDR-TB.^[20] Alagappan *et al.* investigated INH resistance mutations in *M. tuberculosis* in codon *katG* and in the promoter region of the *inhA* gene. One thousand eight hundred and twenty-one (11.8%) of 15,438 INH-resistant strains had detectable mutations: 71.0% in *katG*315 and 29.0% in the *inhA* promoter region.^[21] Isakova *et al.* showed that 91.2% of strains were with *katG* gene mutations, whereas 7% of specimens had *inhA* gene and 2 more specimens (1.8%) had *ahpC* gene mutations.^[22] Jagielski *et al.* also observed that 85.2% of MDR patients had *katG* mutations. About 3.7% of the patients of 54 had single *inhA* mutations, and the rest of the patients, i.e., 11.1%, had both the mutations.^[23] Comparative results are depicted in the following Table 6.

It has been endorsed by the WHO that treating INH-resistance strains with standard anti-TB regimens for new patients resulted in 11% treatment failure and 10% relapses as compared to 2% and 5%, respectively, for

drug-susceptible TB. Acquired drug resistance was also higher (8%) as compared to 1% in drug-susceptible TB. Acquisition of MDR-TB may thus be a serious consequence of the inadequate treatment of INH-resistant TB.

Furthermore, mutations in the *katG* or *inhA* promoter area are relevant to the clinicians.^[24] The presence of mutations in *katG* alone or in combination with *inhA* signifies a high degree of resistance to INH. The addition of even high doses of INH for these patients is unlikely to increase the effectiveness of a regime. A mutation limited only to *inhA*, on the contrary, is usually associated with a low degree of INH resistance, and these individuals are likely to be benefitted with high doses of INH (10–15 mg/kg/day).^[3]

In addition, mutations in *katG* genes have been most frequently associated with *rpoB* gene mutations, making *katG* mutation a better predictor of MDR-TB.^[25] Our study also supports this hypothesis; *katG* mutation was observed in 96 isolates of 106 isolates (90.5%). Therefore, a knowledge about these gene mutations among treating physicians and the value of reporting these mutations by the laboratories cannot be overemphasized.

Our study had limitations in terms of differences in gene mutation patterns among recurrent TB patients and those without any history of anti-TB treatment in the past, as these data were not available for all those patients registered for the study.

CONCLUSION

The study concludes that the most common mutation in INH monoresistance is *katG* 125 (65.1%) as compare to *inhA* 54 (28.1%) and both *inhA* and *katG* 13 (6.7%). Among *katG* mutations, the most common gene pattern is the absence of WT (S315T) and the presence of MUT1 (S315T1), whereas among *inhA* mutations, the most common gene pattern is the absence of WT1 (C15T) and the presence of MUT1. For MDR-TB patients, the most common gene pattern is the absence of WT1 (C15T) and the presence of MUT1 for *inhA*, while it was the absence of WT (S315T) and the presence of MUT1 (S315T1) for *katG*. Our findings are very similar to other reports from India as well as from other countries.

katG also happened to be the most common gene mutant associated with MDR-TB strains.

Since these mutations are very closely related to high- or low-degree resistance to INH and as the therapeutic regimen differs for the two, it is important to know about the gene pattern in each of these patients.

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Conflicts of interest

There are no conflicts of interest.

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