HHS Public Access

Author manuscript

Int J Tuberc Lung Dis. Author manuscript; available in PMC 2017 February 15.

Published in final edited form as:

Int J Tuberc Lung Dis. 2016 August ; 20(8): 1099–1104. doi:10.5588/ijtld.15.0864.

Detection of *katG* and *inhA* mutations to guide isoniazid and ethionamide use for drug-resistant tuberculosis

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SUMMARY

BACKGROUND—Depending on the presence of mutations that determine isoniazid (INH) susceptibility (*katG* and *inhA*), *Mycobacterium tuberculosis* may be susceptible to high doses of INH or ethionamide (ETH).

OBJECTIVE—To describe the INH resistance profile and association of *katG* mutation with previous INH treatment and level of drug resistance based on rapid molecular drug susceptibility testing (DST) in southern Brazil and central Mozambique.

DESIGN—Descriptive study of 311 isolates from Ribeirão Preto, São Paulo, Brazil (2011–2014) and 155 isolates from Beira, Mozambique (2014–2015). Drug resistance patterns and specific gene mutations were determined using GenoType® MTBDR*plus*.

RESULTS—*katG* gene mutations were detected in 12/22 (54.5%) Brazilian and 32/38 (84.2%) Mozambican isolates. *inhA* mutations were observed in 9/22 (40.9%) isolates in Brazil and in 4/38 (10.5%) in Mozambique. Both *katG* and *inhA* mutations were detected in respectively 1/22 (5%) and 2/38 (5.2%). The difference in the frequency of *katG* mutations in Brazil and Mozambique was statistically significant ($P = 0.04$). *katG* mutations were present in 68.8% (33/48) of patients previously treated with INH and 31.2% (15/48) of patients without previous INH. This difference was not statistically significant ($P = 0.223$).

CONCLUSION—INH mutations varied geographically; molecular DST can be used to guide and accelerate decision making in the use of ETH or high doses of INH.

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Conflicts of interest: none declared.

Keywords

multidrug-resistant tuberculosis; INH resistance; molecular diagnosis; line-probe assay; mutations

TUBERCULOSIS (TB) REMAINS one of the most challenging global health problems as resistance to first-line antimycobacterial drugs continues to rise in many countries worldwide. Multidrug-resistant TB (MDR-TB, defined as resistance to at least isoniazid [INH] and rifampicin [RMP]) and extensively drug-resistant TB (XDR-TB, defined as MDR-TB plus resistance to one of the fluoroquinolones and to one of the second-line injectable anti-tuberculosis drugs: amikacin, kanamycin or capreomycin) are increasing globally, with almost 480 000 new MDR-TB cases and 210 000 estimated deaths due to MDR-TB worldwide in 2013.[1]

INH is one of the cornerstones of anti-tuberculosis treatment, as it exhibits mycobactericidal activity by inhibiting mycolic acid biosynthesis.[2] INH resistance commonly occurs due to mutations in the *kat*G gene or the *inh*A regulatory regions. *kat*G encodes catalase peroxidase, an enzyme that converts INH to its biologically active form. As mutations in *kat*G*,* particularly at codon 315, confer high-level INH resistance, INH is ineffective for the treatment of *Mycobacterium tuberculosis* with this mutation profile.[3] The *inh*A regulatory region encodes nicotin-amide adenine dinucleotide-dependent enoyl-acyl carrier protein reductase, the primary target of active INH,[4,5] as well as ethionamide (ETH) and prothionamide (PTH). *inh*A mutations cause low-level resistance to the drug, which means that high doses of INH may be effective against *M. tuberculosis*.[6,7]

As ETH is a structural analogue of INH, cross-resistance occurs between INH and ETH.[8–10] Low-level resistance to INH (*inh*A mutated) can also confer resistance to ETH, while *M. tuberculosis* with high-level INH resistance (*kat*G mutated) is susceptible to ETH. Many studies have demonstrated that high doses of INH (16–18 mg/kg) may be safely used without increased risk of toxicity for the treatment of MDR- and XDR-TB in cases of low-level INH resistance (*inh*A mutated). A randomised clinical trial has reported on the benefits of adding high-dose INH systematically to a standard MDR-TB regimen.[11] ETH is a treatment option in case of MDR- or XDR-TB caused by strains with *kat*G mutations.[10,11]

The frequency of these mutations varies geographically. *kat*G mutations tend to be more frequent (42–95% of isolates), while *inh*A mutations occur in 6–43% of isolates; around 10% of *M. tuberculosis* isolates have both mutations.[4,6,7] Recognition of INH resistance patterns and the frequency of *kat*G and *inh*A mutations in different geographic areas may help to guide decision making about standardisation of treatment regimens or individualised treatment, mainly in the case of MDR- or XDR-TB, as in these settings the number of effective available drugs is limited. We conducted a descriptive study to determine the frequency of *inh*A and *kat*G mutations in two tertiary hospitals that are also TB reference centres, one in southern Brazil and another in central Mozambique.

MATERIALS AND METHODS

Data collection

All the Brazilian and Mozambican patients included in this study were diagnosed with TB based on growth of *M. tuberculosis* in culture. The isolates from the Hospital das Clínicas of the Ribeirão Preto Medical School in Ribeirão Preto, SP, Brazil, were collected from 2011 to 2014; isolates from Beira Central Hospital, Beira, Mozambique, were obtained from the TB Referral Laboratory from 2014 to 2015. Isolates were collected sequentially in both settings.

Clinical samples were collected during routine diagnostic TB investigations according to established standard operational procedures in the hospitals' mycobacteria laboratories: acid-fast bacilli (AFB) examination using Ziehl-Neelsen staining (Brazil) and auramine-rhodamine staining (Mozambique). The specimens were then incubated in liquid medium culture using the BACTEC™ MGIT™ 960 automated system (BD, Sparks, MD, USA). For patients with more than one sample, only the first one was included.

Drug susceptibility testing

Genotypic drug susceptibility testing (DST) was performed using GenoType® MTBDR*plus* version 2.0 (Hain Lifescience, Nehren, Germany), a line-probe assay (LPA) that is able to detect resistance to RMP and INH. The test was performed using only positive liquid culture (MGIT 960). The procedure involved DNA extraction, multiplex amplification with biotinylated primers and DNA reverse hybridisation, according to the manufacturer's instructions. We also assessed mutations in the *kat*G gene, which confers high-level resistance, and the *inh*A regulatory region for low-level resistance.[12,13]

In Brazil, the isolates were tested using non-radiometric phenotypic DST in liquid medium MGIT 960. No information was available on the phenotypic DST performed in the isolates in Mozambique.

Clinical information

All patient records were reviewed, and clinical information, including the previous use of anti-tuberculosis drugs (partial or complete treatment) was collected. In Mozambique, data from patient records were available for only two thirds of the cases studied, and many of these had incomplete information.

Data analyses

Data were analysed using Stata/SE version 13.0 (Stata Statistical Software, Release 13; StataCorp, College Station, TX, USA). Associations between the presence or absence of *kat*G mutations and previous use of INH were determined using Pearson's χ^2 and Fisher's exact tests. $P < 0.05$ was considered statistically significant.

Ethical aspects

The project was approved by the Ethics and Research Committee of the Hospital das Clínicas of the Ribeirão Preto Medical School (Ribeirão Preto, SP, Brazil), the National

Ethical Committee of Ministry of Health of Mozambique (Maputo, Mozambique) and the University of Pittsburgh Institutional Review Board (Pittsburgh, PA, USA).

RESULTS

Of 466 *M. tuberculosis* isolates analysed, 311 were from Brazil and 155 from Mozambique. Of the 311 Brazilian isolates, 22 (7.1%) were INH-resistant, 15 (68.2%) of which were also RMP-resistant (i.e., MDR-TB). The remaining seven (31.8%) were resistant only to INH based on molecular and phenotypic testing. The performance of molecular LPA was compared with that of phenotypic DST in the Brazilian isolates. All of the 22 INH-resistant *M. tuberculosis* cases diagnosed using LPA were also detected by phenotypic DST. However, three INH-resistant isolates were detected by phenotypic DST but not by LPA. Compared with phenotypic DST, LPA sensitivity was 88% (22/25 INH-resistant isolates).

Of the 155 samples from Mozambique, 38 (24.5%) were INH-resistant, 25 (65.8%) of which were also RMP-resistant (i.e., MDR-TB) and 13 (34.2%) were INH-monoresistant based on molecular testing alone.

The *kat*G mutation was detected in 12/22 (54.5%) of the isolates in Brazil and 32/38 (84.2%) of those in Mozambique. The *inh*A mutation was present in 9/22 (40.9%) Brazilian isolates and in only 4/38 (10.5%) Mozambican isolates. Coexistent *kat*G and *inh*A mutations were detected in 1/22 (4.5%) Brazilian and 2/38 (5.3%) Mozambican INH-resistant isolates. The difference in the frequency of *kat*G and *inh*A mutations in isolates from Brazil and Mozambique ($P = 0.04$) was statistically significant (Table 1).

The Figure shows the mutation patterns detected using MTBDR*plus*. S315T *kat*G, with loss of wild-type (wt) mutation, was detected in 75% (45/60) of the isolates, including three with concomitant *inh*A mutation. In *inh*A mutations, C15T with loss of wt1 was detected in 25% (12/60) of the isolates, including two with *kat*G mutation.

The impact of previous use of INH on the mutation profile was analysed by pooling resistant isolates from Brazil and Mozambique. *kat*G mutations were twice as frequent in patients previously treated with INH. The *kat*G mutation was present in 68.8% (33/48) of culture isolates from patients who had been treated at least once with INH for >30 days. The *kat*G mutation was detected in 31.2% of patients without previous treatment with INH (15/48). However, the difference between the two groups was not statistically significant ($P = 0.223$). The proportion of *inh*A mutation in patients with or without previous INH use was the same, at 50% (6/12). The coexistence of *kat*G and *inh*A mutations in the same isolate occurred only in patients who had been previously treated with INH (Table 2).

DISCUSSION

Commercial liquid culture systems and molecular LPAs have been endorsed by the World Health Organization (WHO) as the gold standard for the rapid detection of MDR-TB. However, due to their technical complexity, high cost and the need for sophisticated laboratory infrastructure, the use of these techniques has been limited in many resource-constrained settings.[14] In many countries, such as Brazil and Mozambique, commercial

liquid culture is available only in national or supranational reference laboratories, and DST results can take more than 3–4 months after the clinical specimen has been collected and sent for testing.[15]

New LPAs are easier to perform and more accessible than commercial liquid culture. They also provide important information that can help clinicians initiate suitable treatment for drug-resistant TB more rapidly. As well as providing an accurate diagnosis for MDR-TB, LPAs also provide information about the level of INH resistance, depending on the mutation detected.[16] Katiyar et al. compared high doses of INH (16–18 mg/kg), a normal dose of INH and placebo in a trial of 123 MDR-TB subjects. They found that high doses of INH led to rapid culture negativity of sputum and increased the likelihood of sputum being negative by month 6 of treatment compared with the other two groups. In that trial, all the patients in the high INH dose arm were treated, regardless of the mutation present.[11] Information provided by molecular DST would better identify patients who would benefit from ETH (*kat*G mutations) and those more likely to respond to high doses of INH, avoiding unnecessary exposure to these drugs.[11,17,18]

The frequency of detected *kat*G mutations was higher in Mozambican than in Brazilian isolates (84.2% vs. 54.5%), while that of *inh*A mutations was higher in Brazil than in Mozambique (40.9% vs. 10.5%). According to various studies, the frequency of *kat*G mutations in INH-resistant *M. tuberculosis* may vary between 31.8% and 96.9%.[19–21] Reports from different regions of the world show high variations in the proportion of *kat*G and *inh*A mutations. Despite the high variability, *kat*G mutations are consistently more frequent than *inh*A mutations.[19–32] Table 3 shows data from studies of the two mutations using different molecular strategies in the past two decades. Data from Brazilian studies also showed wide geographic variability within the country. The prevalence of *kat*G mutations varied between 41% and 80.2% in the central western and southern regions.[22,23]

In this study, one third of the isolates showed low-level INH resistance (*inh*A mutation); for these patients, high doses of INH would be a possible option in the management of drug-resistant TB. Seifert et al. reported that the WHO American region had the highest frequency of *inh*A mutations (24%), while in the African region, the frequency tends to be lower.[4] In these regions, high doses of INH could be a therapeutic possibility, while thionamides may be a good option in settings with *kat*G mutations, provided information on drug resistance is available to the clinicians. However, it is important to be aware of the possibility of cross-resistance between low-level INH resistance and ETH, as reported by Schaaf et al. in South Africa[33] and Vadwai et al. in India.[18]

In our study, high-level INH resistance (*kat*G mutations) were more frequent in isolates obtained from patients with a history of previous INH for the treatment of latent tuberculous infection (LTBI) or active TB than in patients without a history of INH (68.8% vs. 31.2%); however, this difference was not statistically significant ($P = 0.223$), probably because of the small number of INH-resistant patients. Mozambique has a higher TB and human immunodeficiency virus (HIV) burden than Brazil,[1] and INH has been used in Mozambique to treat LTBI in all HIV-infected people, while in Brazil the treatment of LTBI is restricted and based on tuberculin skin testing.[34,35] This could be an additional factor contributing to

the high occurrence of *katG* mutations in Mozambique, similar to data from other African countries.[20,24,28]

Most INH-resistant isolates carried S315T in the *katG* gene, with the loss of a wt band, as described by Tolani et al., Maurya et al. and Singhal et al.[25–27] Classical studies have shown that INH-resistant mutants with a defective *katG* gene were catalase-deficient and markedly attenuated in guinea pigs; however, recent studies suggest that INH resistance is not always accompanied by compromised virulence and/or fitness. INH-resistant clinical isolates, particularly S315 mutants from South India, have comparable virulence to that of wt H37Rv, but increased resistance to INH.[33] The most prevalent mutation in the *inhA* gene detected using LPA in our study was C15T, with loss of wt1; this is also supported by previous studies.[21,24,25,28–32]

It is important to recognise the limitations of this study. This was a small case study, and further prospective studies are encouraged. There were gaps due to the poor quality of patient records in both countries, and phenotypic DST results in Mozambican isolates were missing. However, as, according to the WHO,[12] there is high correlation between phenotypic and genotypic DST, we believe this had no influence on our results.

In conclusion, developing countries will probably have greater access to molecular DST before phenotypic testing becomes universal. As evidenced by the geographical variations in the mutations associated with INH resistance, new rapid molecular tests can be used to understand the INH DST profile and to guide treatment decisions.

Acknowledgments

The authors thank M M P Nascimento, R H C Pocente, V Sofia, T Inês, J Beirao and J Ferro for assistance in data collection and performing tests; K Stewart and J M Snyder for assistance with financial documents; and M Sarifa, D L Pakstis, and L A Deluco for assistance with IRB documentation.

The work was funded by the Fogarty International Center of the University of Pittsburgh (Pittsburgh, PA, USA) federal grant 5D43TW001038-15. Fundação de Apoio ao Ensino, Pesquisa e Assistência (Ribeirão Preto, SP) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brasilia DF, Brazil).

References

1. World Health Organization. Global tuberculosis control, 2014. Geneva, Switzerland: WHO; 2014. WHO/HTM/TB/2014.08
2. Takayama K, Wang L, David HL. Effect of isoniazid on the in vivo mycolic acid synthesis, cell growth, and viability of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 1972; 2:29–35. [PubMed: 4208567]
3. Blanchard JS. Molecular mechanisms of drug resistance in *Mycobacterium tuberculosis*. Annu Rev Biochem. 1996; 65:215–239. [PubMed: 8811179]
4. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis*: a systematic review. PLOS ONE. 2015; 10:e0119628. [PubMed: 25799046]
5. Scardigli A, Caminero JA. Management of drug-resistant tuberculosis. Curr Respir Care Rep. 2013; 2:208–217.
6. Zhang Y, Yew WW. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis. 2009; 13:1320–1330. [PubMed: 19861002]

7. Müller B, Streicher EM, Hoek KG, et al. *inh*A promoter mutations: a gateway to extensively drug-resistant tuberculosis in South Africa? Int J Tuberc Lung Dis. 2011; 15:344–351. [PubMed: 21333101]
8. Baulard AR, Betts JC, Engohang-Ndong J, et al. Activation of the pro-drug ethionamide is regulated in mycobacteria. J Biol Chem. 2000; 275:28326–28331. [PubMed: 10869356]
9. Lee H, Cho SN, Bang HE, et al. Exclusive mutations related to isoniazid and ethionamide resistance among *Mycobacterium tuberculosis* isolates from Korea. Int J Tuberc Lung Dis. 2000; 4:441–447. [PubMed: 10815738]
10. Moulding TS. Should isoniazid be used in retreatment of tuberculosis despite acquired isoniazid resistance? Am Rev Respir Dis. 1981; 123:262–264. [PubMed: 6784621]
11. Katiyar SK, Bihari S, Prakash S, Mamtani M, Kulkarni H. A randomised controlled trial of high-dose isoniazid adjuvant therapy for multidrug-resistant tuberculosis. Int J Tuberc Lung Dis. 2008; 12:139–145. [PubMed: 18230245]
12. World Health Organization. Policy statement. Geneva, Switzerland: WHO; 2008. Molecular line-probe assays for rapid screening of patients at risk of multi-drug resistant tuberculosis (MDR-TB). http://www.who.int/tb/features_archive/policy_statement.pdf [Accessed August 2015]
13. Vijdea R, Stegger M, Sosnovskaja A, Andersen AB, Thomsen VO, 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; 27:1079–1086. [PubMed: 18528720]
14. World Health Organization. Policy statement. Geneva, Switzerland: WHO; 2011. Noncommercial culture and drug-susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis. WHO/HTM/TB/2011.9
15. Feliciano CS, Nascimento MM, Anselmo LM, Pocente RH, Bellissimo-Rodrigues F, Bollela VR. Role of a GenoType MTBDR*plus* line-probe assay in early detection of multidrug-resistant tuberculosis at a Brazilian reference center. Braz J Med Biol Res. 2015; 48:759–764. [PubMed: 26132094]
16. Torres JN, Paul LV, Rodwell TC, et al. Novel *kat*G mutations causing isoniazid resistance in clinical *M. tuberculosis* isolates. Emerg Microbes Infect. 2015; 4:e42. [PubMed: 26251830]
17. Van Deun A, Maug AK, Salim MA, et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. Am J Respir Crit Care Med. 2010; 182:684–692. [PubMed: 20442432]
18. Vadwai V, Ajbani K, Jose M, et al. Can *inh*A mutation predict ethionamide resistance? Int J Tuberc Lung Dis. 2013; 17:129–130. [PubMed: 23146620]
19. Telenti A, Honore N, Bernasconi C, et al. Genotypic assessment of isoniazid and rifampin resistance in *Mycobacterium tuberculosis*: a blind study at reference laboratory level. J Clin Microbiol. 1997; 35:719–723. [PubMed: 9041419]
20. Van Rie A, Warren R, Mshanga I, et al. Analysis for a limited number of gene codons can predict drug resistance of *Mycobacterium tuberculosis* in a high-incidence community. J Clin Microbiol. 2001; 39:636–641. [PubMed: 11158121]
21. Hillemann D, Rüsch-Gerdes S, Richter E. Evaluation of the GenoType MTBDR*plus* assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. J Clin Microbiol. 2007; 45:2635–2640. [PubMed: 17537937]
22. Santos LC, de Bousquet HM, Pereira AM, Junqueira-Kipnis AP, Kipnis A. A high prevalence of resistance in new tuberculosis cases of midwestern Brazil. Infect Genet Evol. 2010; 10:1052–1057. [PubMed: 20619356]
23. Perizzolo PF, Dalla Costa ER, Ribeiro AW, et al. Characteristics of multidrug-resistant *Mycobacterium tuberculosis* in southern Brazil. Tuberculosis (Edinb). 2012; 92:56–59. [PubMed: 22001593]
24. Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME. Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. Am J Respir Crit Care Med. 2008; 177:787–792. [PubMed: 18202343]

25. Tolani MP, D'Souza DT, Mistry NF. Drug resistance mutations and heteroresistance detected using the GenoType MTBDR*plus* assay and their implication for treatment outcomes in patients from Mumbai, India. BMC Infect Dis. 2012; 12:9. [PubMed: 22260344]
26. Maurya AK, Singh AK, Kant S, et al. Use of GenoType® MTBDR*plus* assay to assess drug resistance and mutation patterns of multidrug-resistant tuberculosis isolates in northern India. Indian J Med Microbiol. 2013; 31:230–236. [PubMed: 23883707]
27. Singhal R, Myneedu VP, Arora J, et al. Early detection of multidrug resistance and common mutations in *Mycobacterium tuberculosis* isolates from Delhi using GenoType MTBDR*plus* assay. Indian J Med Microbiol. 2015; 33(Suppl):46–52. [PubMed: 25657156]
28. Li Z, Kelley C, Collins R, Rouse D, Morris S. Expression of *kat*G in *Mycobacterium tuberculosis* is associated with its growth and persistence in mice and guinea pigs. J Infect Dis. 1998; 177:1030–1035. [PubMed: 9534978]
29. Ameeruddin NU, Hanna LE. Impact of isoniazid resistance on virulence of global and south Indian clinical isolates of *Mycobacterium tuberculosis*. Tuberculosis (Edinb). 2014; 94:557–563. [PubMed: 25270728]
30. Kiepiela P, Bishop KS, Smith AN, Roux L, York DF. Genomic mutations in the *kat*G, *inh*A and *aph*C genes are useful for the prediction of isoniazid resistance in *Mycobacterium tuberculosis* isolates from KwaZulu Natal, South Africa. Tubercle Lung Dis. 2000; 80:47–56.
31. Zhang Z, Lu J, Liu M, et al. Genotyping and molecular characteristics of multidrug-resistant *Mycobacterium tuberculosis* isolates from China. J Infect. 2015; 70:335–345. [PubMed: 25485999]
32. Cavusoglu C, Turhan A, Akinci P, Soyler I. Evaluation of the GenoType MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* isolates. J Clin Microbiol. 2006; 44:2338–2342. [PubMed: 16825346]
33. Miotto P, Piana F, Penati V, Canducci F, Migliori GB, Cirillo DM. Use of GenoType MTBDR assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* clinical strains isolated in Italy. J Clin Microbiol. 2006; 44:2485–2491. [PubMed: 16825369]
34. Nikolayevskyy V, Balabanova Y, Simak T, Malomanova N, Fedorin I, Drobniewski F. Performance of the GenoType MTBDR*plus* assay in the diagnosis of tuberculosis and drug resistance in Samara, Russian Federation. BMC Clin Pathol. 2009; 9:2. [PubMed: 19284561]
35. Schaaf HS, Victor TC, Venter A, et al. Ethionamide cross- and co-resistance in children with isoniazid-resistant tuberculosis. Int J Tuberc Lung Dis. 2009; 13:1355–1359. [PubMed: 19861006]
36. Ministério da Saúde, Direção Nacional de Assistência Medica. Guia de tratamento antirretroviral e infeções oportunistas no adulto adolescente: gravida e criança. Maputo, Mozambique: MISAU; 2013. [Portuguese]
37. Brazil Ministerio da Sáude, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. Manual de recomendações para o controle da tuberculose no Brasil. Brasília DF, Brazil: Ministério da Saúde; 2011. [Portuguese]

MZB ↓	Mutations observed in the HAIN MTBDRplus® test					Resistance type	
n (%)	*katG* WT	*katG* S315T	*inhA* WT2	*inhA* WT1	*inhA* C15T	MDR-TB	INH-R
30 (78.9%)						20	10
2 (5.3%)						2	0
2 (5.3%)						1	1
1 (2.6%)						1	0
1 (2.6%)						0	1
1 (2.6%)						0	1
1 (2.6%)						1	0
Total = 38						25	13
BRA ↓							
n (%)	*katG* WT	*katG* S315T	*inhA* WT2	*inhA* WT1	*inhA* C15T	MDR-TB	INH-R
12 (54.5%)						8	4
8 (36.4%)						6	2
1 (4.5%)						1	0
1 (4.5%)						1	0
Total = 22						16	6

Figure.

Mutations identified using GenoType® MTBDR*plus* in the *inh*A and *kat*G gene of INH-resistant and MDR-TB strains from Brazil and Mozambique. wt = wild type; MDR-TB = multidrug-resistant tuberculosis; INH-R = isoniazid-monoresistant.

Table 1

INH resistance patterns and frequency of *inh*A and *kat*G mutations in *M. tuberculosis* isolates from Brazil and Mozambique

	MDR-TB			INH-^{R}			Total INH resistance		
	Brazil *n* (%)	**Mozambique *n* (%)**	***P* value***	**Brazil *n* (%)**	**Mozambique *n* (%)**	***P* value***	**Brazil *n* (%)**	**Mozambique *n* (%)**	***P* value***
*inh*A	7 (46.6)	2 (8.0)	0.03	2 (28.6)	2 (15.4)	0.74	9 (40.9)	4 (10.5)	0.04
*kat*G	7 (46.6)	22 (88.0)		5 (71.4)	10 (76.9)		12 (54.5)	32 (84.2)	
*kat*G/*inh*A	1 (6.8)	1 (4.0)		0	1 (7.7)		1 (4.5)	2 (5.3)	
Total	15 (100)	25 (100)		7 (100)	13 (100)		22 (100)	38 (100)	

*Pearson's χ^2 and Fisher's exact test.

INH = isoniazid; MDR-TB = multidrug-resistant tuberculosis; INH-^{R} = INH-monoresistant.

Table 2

Previous use of INH to treat latent tuberculous infection or active TB and rate of detection of *kat*G mutations

	Previous use of INH*						
	Yes			No			
INH-resistant	**Brazil *n* (%)**	**Mozambique *n* (%)**	**Total**	**Brazil *n* (%)**	**Mozambique *n* (%)**	**Total**	***P* value†**
*kat*G mutation							
Yes	10 (69.2)	23 (65.6)	33 (68.8)	4 (30.8)	11 (34.4)	15 (31.2)	0.223
No	4 (50)	2 (50)	6 (50)	4 (50)	2 (50)	6 (50)	
Total			39			21	

*The difference between Brazil and Mozambique data regarding previous use of INH and the presence of *kat*G mutations was not statistically significant ($P = 0.223$).

†Pearson's χ^2.

INH = isoniazid; TB = tuberculosis.

Int J Tuberc Lung Dis. Author manuscript; available in PMC 2017 February 15.

Table 3

Proportions of *katG* and *inhA* mutations in different regions of the world

World region	*katG* mutations %	*inhA* mutations %	*inhA*/*katG* mutations %	INH-resistant isolates tested *n*	Year, reference
Spain	31.80	45.90	1.17	85	1997[19]
Western Cape, South Africa	95	2	NA	43	2001[20]
Germany	96.9	11.1	10.70	65	2007[21]
Brazil (Center-West)	41	23.50	NA	17	2010[22]
Brazil (Southern)	80.2	NA	NA	121	2012[23]
South Africa	64.10	41.90	10.60	114	2008[24]
India	44.20	45.10	10.50	104	2012[25]
India (Northern)	93.30	28.90	NA	45	2013[26]
India (Delhi)	93.20	13.40	6.60	514	2015[27]
KwaZulu-Natal, South Africa	63.30	2.50	21.50	79	2008[28]
China	61.40	17.80	NA	376	2015[29]
Turkey	73	2.7	NA	37	2006[30]
Italy	67.7	32.4	NA	173	2006[31]
Russia	73.5	1.5	23	117	2009[32]
Brazil (Southern)	59	36	4.50	22	2016*
Mozambique	84.20	10.50	5.30	38	2016*

*Data from this study stratified by country. INH = isoniazid; NA = not available.

Int J Tuberc Lung Dis. Author manuscript; available in PMC 2017 February 15.