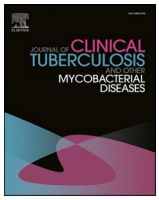
Journal of Clinical Tuberculosis and Other Mycobacterial Diseases 33 (2023) 100387

Contents lists available at ScienceDirect

Journal of Clinical Tuberculosis and Other Mycobacterial Diseases

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



Isoniazid resistance-conferring mutations are associated with highly variable phenotypic resistance

Senamile Lale Ngema [a,1], Navisha Dookie [a,1], Rubeshan Perumal [a,b,1], Louansha Nandlal [a], Nikita Naicker [a], Marothi Peter Letsoalo [a], Max O'Donnell [c], Azraa Khan [a], Nesri Padayatchi [a], Kogieleum Naidoo [a,b,*]

[a] Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa
[b] South African Medical Research Council (SAMRC) – CAPRISA HIV-TB Pathogenesis and Treatment Research Unit, University of KwaZulu-Natal, Durban, South Africa
[c] Division of Pulmonary, Allergy, and Critical Care Medicine, & Department of Epidemiology, Columbia University Medical Center, New York City, NY, United States

ARTICLE INFO

Keywords:
Minimal inhibitory concentration
Isoniazid
Multi-drug resistant tuberculosis
inhA
katG

ABSTRACT

Background: High-dose isoniazid is recommended in the 9–12 months short-course regimen for multidrug-resistant tuberculosis with *inhA* mutation. However, there is insufficient evidence to support the assumption of genotypic-phenotypic concordance. This study aimed to identify the genetic mutations associated with high-level phenotypic isoniazid resistance.

Methods: Clinical isolates from patients with drug-resistant tuberculosis were profiled by whole-genome sequencing and subjected to minimum inhibitory concentration (MIC) testing using MGIT based-method. MICs were performed in concentration ranges based on the mutation present: isolates with no isoniazid resistance-conferring mutations and H37Rv, 0.016–0.256 μg/ml; *inhA,* 0.256–4.0 μg/ml, *katG* 1.0–16.0 μg/ml; and *inhA* + *katG,* 4.0–64.0 μg/ml. Isolates demonstrating resistance at the upper limit of the concentration range were tested up to the maximum of 64.0 μg/ml. Bootstrap of the mean MICs was performed to increase the robustness of the estimates and an overlap index was used to compare the distributions of the MICs for each mutation profile.

Results: A total of 52 clinical isolates were included in this analysis. Bootstrap MIC means for *inhA*, *katG* and *inhA* + *katG* were 33.64 (95% CI, 9.47, 56.90), 6.79 (4.45, 9.70) and 52.34 (42.750, 61.66) μg/ml, respectively. There was high overlap between *inhA* and *inhA* + *katG* mutations (eta = 0.45) but not with *inhA* and *katG* (eta = 0.19). Furthermore, *katG* showed poor overlap with *inhA* + *katG* mutations (eta = 0.09). Unexpectedly, 4/8 (50.0%) of all *InhA* mutants demonstrated high-level resistance, while 20/24 (83.3%) of *katG* mutants demonstrated moderate-level resistance.

Conclusions: InhA mutations demonstrated unexpectedly high MICs and showed high overlap with *inhA* + *katG*. Contrary to the common belief that *katG* mutants are associated with high-level resistance, this mutation primarily showed moderate-level resistance.

1. Introduction

The global burden of multi-drug resistant TB (MDR-TB) continues to rise unabated and poses a significant threat to global TB control efforts [1,2]. There were approximately 500 000 incident cases of rifampicin-resistant TB (RR-TB) reported in 2019, of which 78% were MDR-TB [3]. Isoniazid (INH) resistance was found in 1.4 million drug-resistant TB (DR-TB) cases, 79% of which presented as INH mono-resistant TB [4].

INH resistance in *Mycobacterium tuberculosis* (*M.tb*) is mediated mainly by mutations found in the *inhA* promoter gene (*mabA*), and *katG* genes [5,6]. The S315T mutation in *katG* is the predominant mutation [7,8] present in 40–95% of INH resistant isolates [9,10]. The *katG* S315T mutation is associated with a wide range of moderate to high INH levels of resistance with minimum inhibitory concentrations (MICs) ranging from 2 to > 10 μg/ml [11,12] and is frequently reported in MDR-TB strains [13]. Meanwhile, the most prevalent mutation in the *inhA* promoter region is the C-15T mutation associated with low-level INH

* Corresponding author at: Private Bag X7, Congella, Durban 4001, South Africa.
E-mail address: Kogie.naidoo@caprisa.org (K. Naidoo).
[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.jctube.2023.100387

Available online 26 July 2023

resistance and cross-resistance to ethionamide [12].

Despite their propensity to result in low-level INH resistance, clinical isolates demonstrating *inhA* mutations, have shown to be associated with unfavorable TB treatment outcomes when treated with INH containing regimen [14]. Similarly, isolates displaying *katG* mutations, given their propensity to cause moderate and high-level INH resistance, have been widely associated with unfavorable TB treatment outcomes, but it has not been determined whether this can be overcome with higher doses of INH [14]. Combined mutations in both *inhA* and *katG* result in high-level resistance to INH and are therefore unlikely to be overcome by any clinically permissible dose of INH.

Previous data suggested that patients with low-level INH resistance resulting from *inhA* promoter mutations can still benefit from INH if given at a higher dose (15–20 mg/kg/day) [15]. Furthermore, some studies suggest that patients with moderate-level resistance caused by *katG* mutations may still benefit from high-dose INH [16,17]. On this basis, in its latest report, the World Health Organization (WHO) guidelines on DR-TB recommend high-dose INH as part of the all-oral, bedaquiline-containing, 9–12 months MDR-TB regimen when *inhA* mutations are present alone. Further, the use of high-dose INH in the presence of *katG* mutations is discouraged [18]. The use of high-dose INH in INH mono-resistant TB in the latest report could not be evaluated due to insufficient data [18]. Nonetheless, high-dose INH is discouraged in the presence of *katG* mutation only and when both *inhA* and *katG* co-occur. Moreover, the combination of *inhA* and *katG* mutations, being strongly associated with high-level INH resistance, and conferring cross-resistance to ethionamide disqualifies a patient from receiving the short-course regimen and commits them to the longer and more complex regimen for DR-TB which may be strengthened by the inclusion of high-dose INH. The implications of high-level INH resistance on the critical consideration of regimen selection reflect the importance of definitively establishing the degree to which genotypic resistance can be relied upon to provide an indication of the phenotypic level of resistance.

Leveraging INH for high-dose treatment is appealing, given its potent early bactericidal activity, limited pharmacokinetic interactions, and well-established safety profile. INH displays dose-dependent early bactericidal activity; thus, high doses may result in exposures that overcome resistance mediated through *inhA* and *katG* mutations. Several studies have demonstrated improved time to culture conversion, improved treatment outcomes and successful implementation of an all-oral, short-course treatment, including high-dose INH [19–22].The independent bactericidal effect of high-dose INH on *M. tb* isolates in the presence of *inhA* resistance-conferring mutations has been demonstrated [22]. It was shown that 10–15 mg/kg daily has an activity against *inhA* mutants with media MIC of 1 μg/ml (range 0.05–4 μg/ml) [22]. There is still lack of clinical trial evidence with the use of high-dose INH in the presence of *katG* mutants. We, therefore, aimed to characterize the level of phenotypic resistance associated with each of the INH resistance-conferring mutations identified through whole-genome sequencing (WGS). Furthermore, we compared the distribution of MICs related to the INH resistance-conferring mutations using the estimated overlapping index.

2. Methods

2.1. Study design

This study was performed on stored clinical isolates obtained from CAPRISA 020 participants. The CAPRISA 020 study (ClinicalTrials.gov Identifier: NCT03237182) is an ongoing randomized controlled trial assessing the effectiveness of an individualized, WGS-guided treatment regimen for DR-TB. Adult participants with pulmonary DR-TB were randomized to a standard of care regimen or a WGS-guided regimen as part of the study procedures, and the following tests were performed: Xpert MTB/RIF, line probe assay (LPA), drug susceptibility testing (DST) at critical concentrations (CC), and WGS (WGS-guided participants only). Additionally, culture DST for treatment response monitoring was performed in accordance with WHO guidelines [23].

2.2. Mycobacterium tuberculosis clinical isolates

A total of 94 *M. tb* clinical isolates were requested from the study specimen biorepository. Isolates were selected based on the presence of INH resistance-conferring mutations at >50% frequency identified through pre-existing WGS data from CAPRISA 020, which was performed on clinical isolate as described previously [24]. In addition, control isolates with no mutations linked to isoniazid resistance were also selected. The 94 clinical isolates were derived from screening, enrollment, and follow-up sputum culture specimens of 65 participants. Twenty-one isolates had *inhA* promoter gene and/or *inhA* coding region mutations, 36 had *katG* S315T mutation, 20 had both *inhA* promoter and or *inhA* coding region and *katG* mutations, and 17 isolates had no canonical INH resistance-conferring mutations (selected as control clinical isolates). H37Rv was added as a reference strain.

2.3. Minimal inhibitory concentration (MIC) testing

2.3.1. Growth of clinical isolates

Each clinical isolate was grown in a Mycobacteria Growth Indicator Tube (MGIT) supplemented with BD BBL Middlebrook OADC (Oleic acid, Albumin, Dextrose, catalase) enrichment and incubated in the BACTEC MGIT 960 (Becton Dickinson (BD), New Jersey, USA) instrument until positivity was detected. Isolates that did not show any growth after 42 days (protocol length) were recorded as non-growing and were excluded. Quality control on a positive MGIT tube was done using TBc identification test/MPT64 antigen test, Kinyoun staining and subculturing in Trypticase Soy Agar (TSA) + 5% sheep blood to detect contamination.

2.3.2. MIC testing

The stock concentrations of INH (Media mage Company Pty (Ltd), Johannesburg, South Africa) to make the final concentration of 0.016, 0.032, 0.064, 0.128, 0.256, 0.512, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 μg/ml in MGIT were used. MICs were done in serial two-fold dilutions to provide a final test range of 0.016–0.256 μg/ml for INH susceptible isolates, 0.256–4.0 μg/ml for *inhA* promoter mutant isolates with/without a concurrent *inhA* coding mutation, 1.0–16.0 μg/ml for *katG* mutant isolates, 4.0–64.0 μg/ml for isolates with both *inhA* and *katG* mutations. Those isolates that showed resistance at the upper limit within their concentration range were tested outside the set range up to the maximum of 64.0 μg/ml.

2.3.3. MIC determination

The determination of MICs was performed within the epicenter/TB Exist and MGIT 960 instrument [2,16]. The minimum and the maximum days of the protocol were four and 24 days, respectively, and the minimum number of growth units (GU) for the control was 400. A growth unit of 0–99 was considered susceptible, and ≥ 100 was deemed resistant [2]. In a series of two-fold concentrations tested, the first concentration with a growth unit less than 100 was recorded as the MIC. MICs were further categorized into four resistance groups: Susceptible, MIC ≤ 0.1 μg/ml; Low level resistance, 0.1 < MIC ≤ 1 μg/ml; moderate-level resistance, 1 < MIC ≤ 8 μg/ml; high-level resistance, MIC > 8 μg/ml.

2.4. Data analysis

Descriptive statistics presented in a demographics table were analyzed using IBM SPSS Statistics for Windows, Version 27. The MIC data was analyzed using R software and R markdown was used to document the data manipulation and statistical analysis performed. The goodness of fit test was used to analyze genetic mutations associated with high-level phenotypic INH resistance. To characterize the MICs

obtained for each mutation including INH susceptible group and the distribution of raw MICs for the three resistance mutation groups, basic statistics [minimum, median (interquartile range (IQR), mean (standard deviation), and maximum] was performed. Clinical isolates that had MICs greater > 64.0 μg/ml, the MIC value of 65.0 μg/ml was assumed for statistical analysis purpose. Given the unexpected small sample size within the groups, 1000 non-parametric bootstraps of the means to obtain the 95% confidence intervals (CI) of statistics measures was performed. We assessed the distribution of the MICs of the three mutation groups overlap using the estimated overlapping index ($0 < \widehat{\eta} < 1$), with $\widehat{\eta}$ closer to 0 indicating non-significant overlap while $\widehat{\eta}$ closer to 1 indicating a significant overlap.

3. Results

3.1. Patient demographic and clinical characteristics

The mean age was 35 ± 10 years, 23 (44.2%) participants were male, and 37 (71.2%) participants were HIV co-infected, of whom 27 (73%) were receiving antiretroviral therapy (ART), 63.5% were MDR-TB (Supplementary Table S1). Sixteen (30.8%) participants had a previous history of TB; 28 (53.8%) and 23 (44.2%) participants had unilateral and bilateral disease on chest radiography, respectively, while one (2.0%) participant showed clear chest radiography.

3.2. Mycobacterium tuberculosis clinical isolates

A total of 94 *M. tb* clinical isolates from 65 patients were selected for this study, of which 42 clinical isolates were excluded: 11 isolates negative for MPT64 antigen and demonstrating non-roping acid-fast bacilli, 23 non-growing isolates, two contaminated isolates and six follow-up isolates. The final analysis included 52 isolates from 52 patients (47 isolates were of screening and five isolates were obtained at enrollment). A patient with no isolate stored for screening, enrollment isolate was selected. Isolates were further stratified based on INH mutation pattern (Fig. 1).

3.3. Phenotypic resistance to isoniazid

The MICs of clinical isolates from this cohort are grouped according to INH resistance-conferring mutations and are presented by means and medians (**Supplementary Table S2**). Isolates with no mutations linked to INH resistance showed mean (SD) MIC of 0.016 (0.34) μg/ml that was within the expected concentration range. *InhA* mutants had a mean MIC of 33.19 (34.03) μg/ml, indicating high-level phenotypic resistance to INH. *KatG* mutants had a mean MIC that was at the lower limit of the expected range, 6.71 (6.73) μg/ml, while the combination of *inhA* and/*inhA* promoter region and *katG* demonstrated high mean MIC, 52.25 (18.40) μg/ml.

The bootstrap means of MICs and the 95% CI for each INH resistant-conferring mutation and INH susceptible group is displayed in **Supplementary** Fig. 1. The mean MIC for *inhA*, *katG* and *inhA* + *katG* were 33.64 (95% CI, 9.47, 56.90), 6.79 (4.45, 9.70) and 52.34 (42.750, 61.66) μg/ml, respectively.

3.4. Association between isoniazid-resistant mutations and phenotypic resistance level

High-confidence mutations were universally associated with phenotypic resistance (Table 1). The high confidence mutations detected were *inhA* promoter region C-15T alone (n = 8), the *katG* Ser315Thr alone (n = 24), the combination of *katG* Ser315Thr + *inhA* promoter region C-15T (n = 3), and *katG* Ser315Thr + *inhA* promoter region T-8A

Table 1
INH susceptible and resistant associated mutation isolates (n = 52) detected from CAP020 cohort of 52 participants. Susceptible MIC ≤ 0.1 μg/ml, Low level resistance 0.1 < MIC ≤ 1 μg/ml; moderate-level resistance 1 < MIC ≤ 8 μg/ml; high-level resistance: MIC > 8 μg/ml.

Mutation	N	Susceptible (%)	Low (%)	Moderate (%)	High (%)
INH Susceptible [#]	8	7 (87.5)	1 (12.5)	0 (0.0)	0 (0.0)
InhA promoter region C-15 T alone	8	0 (0.0)	3 (37.5)	1 (12.5)	4 (50.0)
KatG Ser315Thr alone	24	0 (0.0)	1 (4.2)	20 (83.3)	3 (12.5)
KatG Ser315Thr + inhA promoter region C-15 T	3	0 (0.0)	0 (0.0)	0 (0.0)	3 (100)
KatG Ser315Thr + inhA promoter region T-8A[*]	9	0 (0.0)	0 (0.0)	0 (0.0)	9 (100)

[#] One isolate was drug-susceptible, six rifampicin mono-resistant, and one resistant to rifampicin, ethambutol, and streptomycin.
[*] *InhA* promoter region T-8A mutation did not occur alone, it only co-occurred with *katG* Ser315Thr.

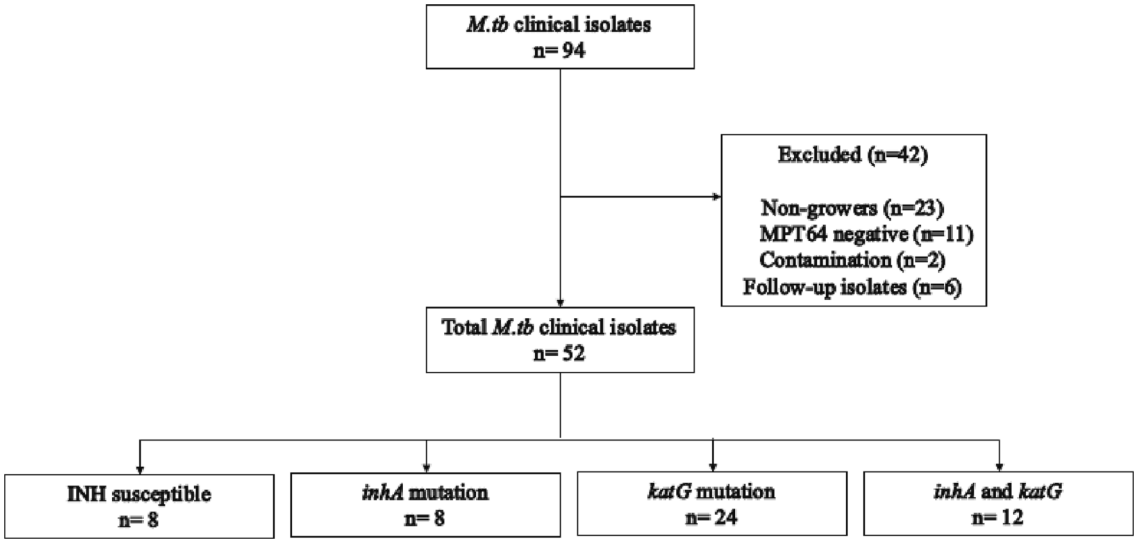


Fig. 1. *Mycobacterium tuberculosis* clinical isolates included in this analysis by INH resistance pattern (*M. tb*: Mycobacterium tuberculosis; INH: isoniazid).

(n = 9). The *inhA* promoter region C-15T mutation alone was associated with highly variable levels of phenotypic resistance (0.256 to > 64 μg/ml), with most isolates displaying high-level resistance. Low-level resistance was exhibited by 3/8 (37.5%), moderate-level resistance by 1/8 (12.5%) and high-level resistance by 4/8 (50.0%). An independently run experiment, which was performed in triplicate, confirmed the MIC findings for *inhA* mutation. The *katG* Ser315Thr mutation alone was associated primarily with moderate and high-level phenotypic resistance (1 to 32 μg/ml), exhibited by 20/24 (83.3%) and 3/24 (12.5%) respectively; low-level resistance was seen in 1/24 (4.2%) of the isolates. Lastly, 100% of isolates with the combination of *katG* Ser315Thr + *inhA* promoter region C-15T and *katG* Ser315Thr + *inhA* promoter region T-8A mutations were universally associated with high-level phenotypic resistance (>8 μg/ml).

3.5. MIC overlapping distribution

The distribution of MICs for INH susceptible and INH resistant-conferring mutations is shown in Fig. 2. The distribution of MICs related to the paired INH resistance-conferring mutations using the estimated overlapping index is shown in **Supplementary** Fig. 2. *InhA* mutation showed high overlap with *inhA* + *katG* mutations (eta = 0.45) and showed less overlap with *katG* (eta = 0.19). Furthermore, *katG* showed poor overlap with *inhA* + *katG* mutations (eta = 0.09).

Supplementary Table S3 presents the summary of INH mutation profile, INH MIC raw data, additional TB drug resistance, past TB history, DR-TB treatment regimen, and week eight culture conversion data for the 52 participants.

4. Discussion

We observed a wide range of MICs in clinical isolates, with a high overlap of MICs among the most widely described INH mutations. Genotypic resistance was associated with highly variable phenotypic resistance. Although widely associated with low-level phenotypic resistance, we found that the most identified INH resistance genotype, *inhA* mutation, can be associated with moderate- and high-level resistance. High-level resistance was displayed by a high proportion of the isolates in the *inhA* group in this study.

Although the study is limited by its sample size, the findings were strengthened by a 1000 sample non-parametric bootstrap. We found MICs for *inhA* mutants to be far higher than previously reported and strikingly similar to MICs observed for double mutants (*inhA* + *katG*). The overlapping estimate showed the high overlap between *inhA* and *inhA* + *KatG* mutations. This finding departs from prior studies of the genotypic-phenotypic correlation, which found *inhA* mutations to be universally associated with low phenotypic resistance [2,11,12,25,26]. Previous studies have consistently reported an MIC ≤ 4.0 μg/ml for mutations in the *inhA* gene [11,25,27,28]. Based on the historical findings, the WHO DR-TB treatment guideline continues recommend high-dose INH to overcome the presumed low-level resistance in DR-TB cases with *inhA* mutation [29]. Futile exposure to INH in patients with *inhA* mutations may constitute an unnecessary and avoidable risk for toxicity. However, a major challenge is that front-line diagnostic assays used to detect resistance to INH do not provide sufficient information to accurately predict the phenotypic level of resistance. LPA only reports on the presence/absence of the mutation (*inhA* and/*katG*), and these results are commonly interpreted as *inhA*: low-level resistance and *katG*: high-level resistance. Culture-based phenotypic DST is done at a CC of 0.1 μg/ml (INH low) and a clinical breakpoint of 0.4 μg/ml (INH high) [30,31]. Both concentrations used to define low and high-level resistance, respectively, are insufficient to identify clinically important thresholds for determining the likelihood of response to high-dose INH. Furthermore, the WHO has not established the concentration/s that would define the moderate level resistance.

MICs of *KatG* mutants were in keeping with published reports [25,26,32,33]. As expected, there was no high overlap observed between *katG* and *inhA* mutations. This expectation, however, was based on *inhA* mutants demonstrating low-level phenotypic resistance and *katG* mutants demonstrating high-level resistance. To the contrary, in this study this was due to *inhA* MICs being far higher than in previous reports and *katG* mutation MICs being lower than expected (mostly moderate level phenotypic resistant). The combination of *InhA* and *katG* mutants demonstrated high-level phenotypic resistance, consistent with previous studies [12,25,28].

InhA and *katG* mutants with MICs below a serum peak of 15 μg/ml may potentially be overcome by high-dose INH (15–20 mg/kg), provided that the patient is on a well-constructed regimen [34,35]. In a

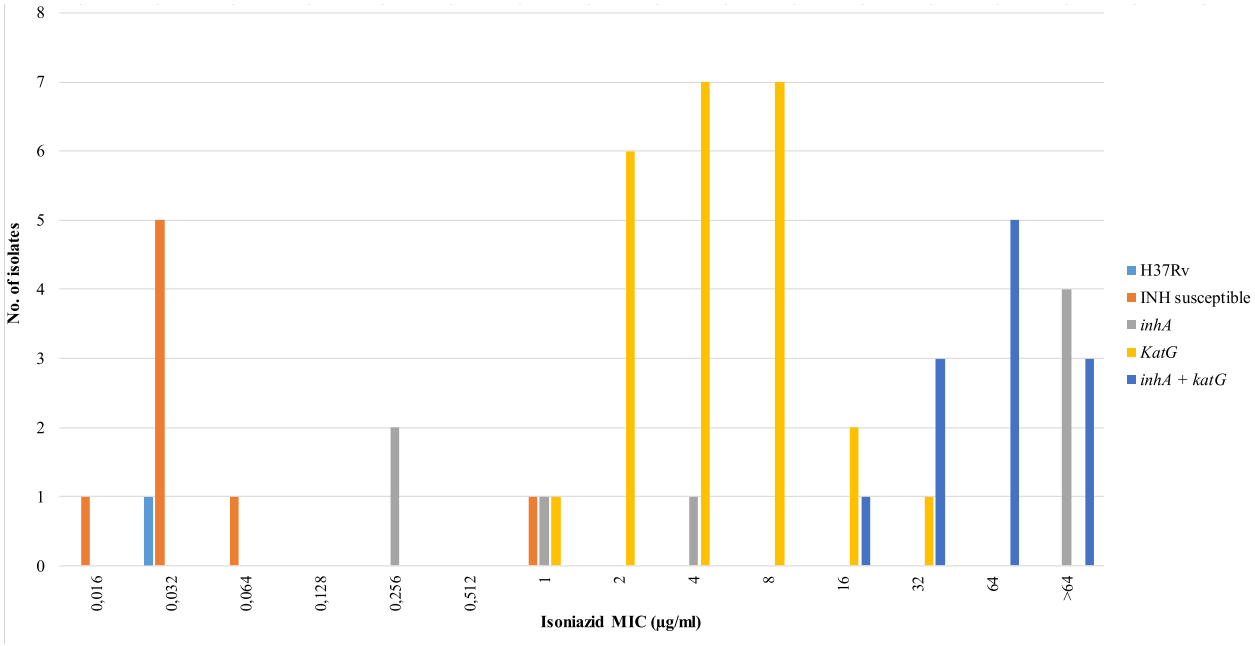


Fig. 2. Isoniazid resistant clinical isolates MIC distribution. MICs are presented separately for the control strain H37Rv, INH susceptible, *inhA* mutation, *katG* mutation and *inhA* + *katG* mutations. (Abbreviation: INH, isoniazid; MIC, minimum inhibitory concentration).

clinical trial, it has been demonstrated that 10–15 mg/kg daily of INH has activity against *inhA* mutants with a median INH MIC of 1 μg/ml (range 0.05–4 μg/ml) [22]. This study highlighted the importance of MICs as a guide in identifying patients that can benefit from high-dose INH. Moreover, MIC data obtained from the current study does not support the assertion that *inhA* mutations are universally associated with low-level resistance, as reported previously [34]. Furthermore, INH resistance-conferring mutations cannot accurately predict MICs, hence they cannot reliably inform the choice to include high-dose INH. Our data further demonstrate that *katG* mutants can possibly benefit from high-dose INH as majority demonstrates moderate-level resistance. The efficacy of high-dose INH against *katG* mutants is currently being investigated (NCT01936831).

Our study has several limitations. First, the sample size was small, especially for the *inhA* group. We were not able to perform MIC replicates for all groups due to the high costs and infrastructure requirements associated with such testing. However, MICs were confirmed in triplicate for *inhA* group and the robustness of our estimates were strengthened by bootstrapping. Second, among the isolates included, there were no low-confidence mutations, thus preventing the comparison of phenotypic resistance levels between high- and low-confidence mutations. There was no data on strain lineage which could possibly explain the differences in MICs. Lastly, the current results (MICs only) do not provide sufficient information to guide therapeutic decision-making with high-dose INH. However, we do demonstrate the value that MIC testing may contribute to optimizing efficacy and minimizing toxicity. MICs, together with genotype, individual acetylation status, and pharmacokinetics, may provide more robust data to guide the selection of the optimal dose for INH mutants. Despite these limitations, this study has important strengths such as the use of clinical isolates, representation of isolates from people living with HIV/AIDS, and combination of genotypic and phenotypic data. Additionally, we used validated laboratory methods with stringent standard operating protocols within a well-established laboratory.

5. Conclusion

InhA mutations may not always be associated with low-level resistance, and *katG* mutations may not reliably represent high-level resistance. Thus, genotypic data and DST conducted at a critical concentration or clinical breakpoint cannot be used confidently to anticipate the level of phenotypic resistance.

Ethics approval and consent to participate

Ethics approval for the InDEX (CAPRISA 020) and present sub-study was provided by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BFC584/16 and BREC/00001449/2020). Written informed consent for study participation and sample storage was obtained from all participants. Culture DST for treatment response monitoring was performed in accordance with WHO guidelines.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request (Kogie.naidoo@caprisa.org).

Funding

This work was supported by the National Research Foundation of South Africa [TTK1902114157860, 146343], the Centre of Excellence for Biomedical Tuberculosis Research [TMA2018CDF-2372], the European and Developing Countries Clinical Trials Partnership Fellowships [TMA2018SF-2476] and the National Research Foundation Centre of Excellence [IUD: 146343].

Declaration of Competing Interest

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

Acknowledgements

We would like to acknowledge the participants of the study, the CAPRISA Springfield research clinic, the CAPRISA laboratory and the University of KwaZulu-Natal.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jctube.2023.100387.

References

[1] Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respir Med 2017;5(4):291–360.
[2] Machado D, Perdigão J, Portugal I, Pieroni M, Silva P, Couto I, et al. Efflux Activity Differentially Modulates the Levels of Isoniazid and Rifampicin Resistance among Multidrug Resistant and Monoresistant *Mycobacterium tuberculosis* Strains. Antibiotics 2018;7(1):18.
[3] World Health Organization, "Global tuberculosis report 2020," https://www.who.int/publications/i/item/9789240013, 2020.
[4] World Health Organization, "WHO consolidated guidelines on tuberculosis rapid diagnostics for tuberculosis detection; Module 3: Diagnosis," https://www.who.int/publications/i/item/9789240029, 2021.
[5] Ramaswamy SV, Reich R, Dou S-J, Jasperse L, Pan Xi, Wanger A, et al. Single nucleotide polymorphisms in genes associated with isoniazid resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2003;47(4):1241–50.
[6] M.H. Hazbón *et al.*, "Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant *Mycobacterium tuberculosis*," *Antimicrob. Agents Chemother.*, vol. 50, no. 8, pp. 2640-2649. doi.org/10.1128/aac.00112–06, Aug. 2006.
[7] Stagg HR, Lipman MC, McHugh TD, Jenkins HE. Isoniazid-resistant tuberculosis: a cause for concern? Int J Tuberc Lung Dis 2017;21(2):129–39.
[8] I.D. Purkan *et al.*, "Mutation of *katG* in a clinical isolate of *Mycobacterium tuberculosis*: Effects on catalase-peroxidase for isoniazid activation," *Ukr. Biochem. J.*, vol. 88, no. 5, pp. 71-81. doi.org/10.15407/ubj88.05.071, 2016.
[9] J.M. Musser *et al.*, "Characterization of the Catalase-Peroxidase Gene (*katG*) and *inhA* 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.*, vol. 173, no. 1, pp. 196-202. doi.org/10.1093/infdis/173.1.196, 1996.
[10] I. Mokrousov *et al.*, "High Prevalence of *KatG* Ser315Thr Substitution among Isoniazid-Resistant *Mycobacterium tuberculosis* Clinical Isolates from Northwestern Russia," *Antimicrob. Agents Chemother.*, vol. 46, no. 5, pp. 1417-1424. doi.org/10.1128/aac.46.5.1417-1424.2002, 2002.
[11] Cambau E, Viveiros M, Machado D, Raskine L, Ritter C, Tortoli E, et al. Revisiting susceptibility testing in MDR-TB by a standardized quantitative phenotypic assessment in a European multicentre study. J Antimicrob Chemother 2015;70(3):686–96.
[12] Kambli P, Ajbani K, Sadani M, Nikam C, Shetty A, Udwadia Z, et al. Defining multidrug-resistant tuberculosis: Correlating GenoType MTBDR*plus* assay results with minimum inhibitory concentrations. Diagn Microbiol Infect Dis 2015;82(1):49–53.
[13] Rozwarski DA, Grant GA, Barton DHR, Jacobs WR, Sacchettini JC. Modification of the NADH of the isoniazid target (*InhA*) from *Mycobacterium tuberculosis*. Science 1998;279(5347):98–102.
[14] M.N.T. Huyen *et al.*, "Epidemiology of isoniazid resistance mutations and their effect on tuberculosis treatment outcomes," *Antimicrob. Agents Chemother.*, vol. 57, no. 8, pp. 3620-3627. doi.org/10.1128/aac.00077–13, 2013.
[15] World Health Organization, "Global tuberculosis report 2018," https://www.who.int/publications/i/item/9789241565, 2018.
[16] P. Lempens *et al.*, "Isoniazid resistance levels of *Mycobacterium tuberculosis* can largely be predicted by high-confidence resistance-conferring mutations," *Sci. Rep.*, vol. 8, no. 1, pp. 1-9. doi.org/10.1038/s41598-018-21378–x, 2018.
[17] E. Heldal, "A *katG* 315 mutation alone should not lead to exclusion of isoniazid in treatment of multidrug-resistant tuberculosis," *Eur. Respir. J.*, vol. 50, no. 4, pp. 1701696. doi: 10.1183/13993003.01696–2017, 2017.
[18] World Health Organization, "WHO consolidated guidelines on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment, 2022 update," https://www.who.int/publications/i/item/9789240063, 2022.

[19] Van Deun A, Maug AKJ, Salim MAH, Das PK, Sarker MR, Daru P, et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. Am J Respir Crit Care Med 2010;182(5):684–92.
[20] Katiyar SK, et al. A randomised controlled trial of high-dose isoniazid adjuvant therapy for multidrug-resistant tuberculosis. Japanese J Chest Dis 2010;69(2): 172–3. PMID: 18230245.
[21] K.F. Walsh *et al.*, "Improved Outcomes with High-dose Isoniazid in Multidrug-resistant Tuberculosis Treatment in Haiti," *Clin Infect Dis*, vol. 69, no. 4, pp. 717-719. doi: 10.1093/cid/ciz039, 2019.
[22] Dooley KE, et al. Early bactericidal activity of different isoniazid doses for drug-resistant tuberculosis (INHindsight): A randomized, open-label clinical trial. Am J Respir Crit Care Med Jun. 2020;201(11):1416–24.
[23] World Health Organization, "WHO consolidated guidelines on tuberculosis: Module 4: Treatment - Drug-resistant tuberculosis treatment," https://www.who.int/publications/i/item/9789240007, 2020.
[24] Gautam SS, Kc R, Leong KWC, Mac Aogáin M, O'Toole RF. A step-by-step beginner's protocol for whole genome sequencing of human bacterial pathogens. J Biol Methods 2019;6(1):e110.
[25] Ghodousi A, et al. Isoniazid resistance in *Mycobacterium tuberculosis* is a heterogeneous phenotype comprised of overlapping MIC distributions with different underlying resistance mechanisms. Antimicrob Agents Chemother 2019; 63(7):e00092–119.
[26] Gygli SM, Keller PM, Ballif M, Blöchliger N, Hömke R, Reinhard M, et al. Whole-Genome Sequencing for Drug Resistance Profile Prediction in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2019;63(4). https://doi.org/10.1128/AAC.02175-18, Apr.
[27] Charoenpak R, et al. Association Between the Phenotype and Genotype of Isoniazid Resistance Among *Mycobacterium tuberculosis* Isolates in Thailand. Infect Drug Resist 2020;13:627–34. https://doi.org/10.2147/IDR.S242261, Feb.
[28] Miotto P, Tessema B, Tagliani E, Chindelevitch L, Starks A, Emerson C, et al. A standardised method for interpreting the association between mutations and phenotypic drug resistance in *Mycobacterium tuberculosis*. Eur Respir J 2017;50(6): 1701354.
[29] World Health Organization, "WHO consolidated guidelines on drug-resistant tuberculosis treatment.," https://apps.who.int/iris/handle/10665/311389, 2019.
[30] World Health Organization, "Technical Report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine)," https://apps.who.int/iris/handle/10665/339275, 2021.
[31] Clinical and Laboratory Standards Institute. Susceptibility testing of susceptibility testing of mycobacteria, Nocardia spp., and other aerobic actinomycetes—3rd ed CLSI standard M24. Wayne: Clinical and Laboratory Standards Institute; 2018.
[32] Andres S, Hillemann D, Rüsch-Gerdes S, Richter E. Occurrence of *rpoB* Mutations in Isoniazid-Resistant but Rifampin-Susceptible *Mycobacterium tuberculosis* Isolates from Germany. Antimicrob Agents Chemother 2014;58(1):590–2.
[33] Sturegård E, Ängeby KA, Werngren J, Juréen P, Kronvall G, Giske CG, et al. Little difference between minimum inhibitory concentrations of *Mycobacterium tuberculosis* wild-type organisms determined with BACTEC MGIT 960 and Middlebrook 7H10. Clin Microbiol Infect 2015;21(2):148.e5–7.
[34] Rieder HL, Van Deun A. Rationale for high-dose isoniazid in the treatment of multidrug-resistant tuberculosis. Int J Tuberc Lung Dis 2017;21(1):123–4.
[35] Bollela VR, et al. Detection of *katG* and *inhA* mutations to guide isoniazid and ethionamide use for drug-resistant tuberculosis. Int J Tuberc Lung Dis 2016;20(8): 1099–104.