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Prevalence of *katG* and *inhA* mutations associated with isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates in Cameroon

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Abstract

Background The acquisition of isoniazid (INH) resistance is alarming, considering its importance as a key drug that forms the core of multidrug treatment regimens for tuberculosis (TB). Genetic mutations in the *katG* and *inhA* promoter regions play crucial roles in INH resistance, but their prevalence varies geographically. This study aimed to identify the most common mutations in the *katG* and *inhA* genes in INH-resistant (INH-R) *Mycobacterium tuberculosis* (MTB) clinical isolates in Cameroon. The research also explored the relationships between these mutations and patients' demographics (age, sex, and sample type).

Methods We conducted a retrospective cross-sectional laboratory-based study on 500 INH-R isolates (with or without resistance to other first-line drugs) at the National Tuberculosis Reference Laboratory (NTRL) in Cameroon between January 2014 and December 2020. GenoType MTBDR*plus* assay was performed on the retrieved isolates and the frequency of *katG and inhA* mutations were calculated. Chi-square tests were utilized to assess the associations between these mutations and patients' age, sex and sample type.

Results A total of 410 (85.8%) culture-positive MTB isolates were analyzed, with a male-to-female ratio of 228 (55.6%) to 182 (44.4%) and an average age of 36.3 ± 13.4 years. Mutations in the *katG* and *inhA* genes were detected in 354 (86.3%) of cases, while 56 (13.7%) showed no mutations. Among the INH-R isolates, mutations in *katG*, *inhA*, and dual *katG* and *inhA* genes were present in 247 (60.2%), 76 (18.5%), and 31 (7.6%) isolates, respectively. Our analysis revealed significant associations between mutation prevalence and patient characteristics.

Conclusion This study reaffirmed the importance of the *katG* S315T substitution as a key indicator of INH resistance, with the *inhA* C-15T mutation providing additional support. However, a notable proportion of isoniazid-resistant isolates did not exhibit these mutations, underscoring the need to comprehend resistance mechanisms. Given that these mechanisms are strongly associated with varying levels of INH resistance, it is crucial that TB management

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strategies incorporate genetic profiling alongside patient demographics to optimize treatment outcomes and enhance control measures.

Keywords *Mycobacterium tuberculosis*, Isoniazid resistance, *katG*, *inhA*, Mutations, Cameroon

Background

Tuberculosis (TB), which is predominantly caused by *Mycobacterium tuberculosis* (MTB), remains a significant global public health concern [1]. According to the World Health Organization's (WHO) Global Tuberculosis Report of 2024, there were 8.2 million TB cases and 1.09 million deaths in 2023. This marks TB's return as the world's leading cause of death from a single infectious agent, having been surpassed by coronavirus disease (COVID-19) for the previous three years. In Cameroon, 25,705 TB cases were reported in 2023 with an estimated 12,000 death cases [2]. The emergence of drug-resistant TB, particularly multidrug-resistant TB (MDR-TB), defined as resistance to isoniazid (INH) and rifampicin (RIF), continues to be a significant public health threat globally [2]. In 2023, a total of 159,684 and 28,982 cases globally and 180 and 1 cases in Cameroon were identified as multidrug-resistant/rifampicin-resistant TB (MDR-TB/RR-TB) and pre-XDR-TB or XDR-TB respectively [2]. However, the increasing prevalence of MTB isolates resistant to INH but susceptible to RIF, termed INH-monoresistant TB, affects 8% of global TB cases and, as such, jeopardizes the effectiveness of TB treatment among patients following the standard 6-month first-line regimen [3]. This is supported by the findings of Dean and colleagues, who reported a global prevalence of INH resistance (7.4%) in new cases comparable to that of MDR/RR-TB (3.4%) [4].

INH is an important first-line anti-TB drug that serves as a cornerstone in multidrug regimens for the treatment of TB [5] and for patients infected with RIF-susceptible strains [6] and MDR-TB [7]. INH functions as a prodrug that is activated by the mycobacterial catalase-peroxidase enzyme, encoded by *katG*. Activated INH targets the enzyme enoyl acyl carrier protein reductase encoded by the *inhA* gene, which is essential for mycolic acid biosynthesis [5–8]. The acquisition of resistance to INH is particularly concerning, as it is a key drug in the treatment of both active and latent TB and is highly recommended for HIV-infected individuals at risk of developing active TB [9]. In addition, resistance to INH is a crucial step in the development of MDR-TB [10]. Resistance to INH involves a complex set of genes [11], with mutations at codon 315 of the *katG* gene and the regulatory region of the *mabA-inhA* operon being the main contributors, accounting for 40%–95% and 20%–42% of phenotypic resistance in clinical TB isolates respectively [12]. Mutation at these two gene loci results in a decrease in or complete loss of catalase-peroxidase activity and structural changes in the INH target [13]. In addition to chromosomal mutations at drug target genes and regulatory regions as the predominant cause of drug resistance in MTB [14], recent research has shed light on a phenomenon called heteroresistance, which plays a major step in the development of drug resistance in bacterial isolates [15]. It is not uncommon in MTB, as strains resistant to INH, RIF, ethambutol, and streptomycin (STR) have been reported to exhibit it [16]. Heteroresistance can emerge when both susceptible and resistant strains coexist within a bacterial isolate in a subpopulation, or when a single isolate transitions from susceptibility to resistance due to genetic mutations under antibiotic pressure [12, 15].

Understanding the mechanisms underlying drug resistance is vital for prompt diagnosis and effective treatment. In Cameroon, the predominant approach for detecting drug resistance in MTB isolates involves a combination of rapid molecular methods and phenotypic drug susceptibility testing (DST). A study by Abanda et al., [17] highlighted the effectiveness of the GenoType MTBDR*plus* assay, a rapid molecular diagnostic test, in accurately diagnosing resistance to RIF, INH, and MDR-TB in the country. This assay specifically targets "canonical" mutations at the *katG* gene (S315T) and in the *inhA* promoter region (C-15 T, A-16G, T-8C, and T-8A) associated with INH resistance [17]. Research has shown that the distribution of mutations associated with INH resistance varies with population, geographical location, and genotype [18]. Owing to regional variations in the frequencies of mutations conferring resistance to INH, which may limit the sensitivity of molecular tests for detecting resistance, it is crucial to establish the mutation profiles and frequencies in the specific regions where these tests are implemented [19]. Understanding the patterns of INH resistance and the prevalence of *katG* and *inhA* mutations across various regions is crucial for understanding the epidemiology of the disease and thus, guide clinicians in determining whether treatment regimens should be standardized or personalized, especially in scenarios involving INH-monoresistant or MDR-TB. Thus far, reports on the prevalence of common mutations in the *katG* and *inhA* genes associated with INH-resistant (INH-R) MTB isolates in Cameroon are limited. Hence, we conducted this study to investigate the prevalence of mutations in the two most reported loci (*katG* codon 315

and the *fabG1-inhA* regulatory region) associated with INH resistance as well as analyze if INH resistance and/or mutations are associated with patients' demographics and clinical variable (age, sex and sample type).

Methods

Study design, area and period

This was a retrospective cross-sectional laboratory-based study. All laboratory activities were performed in the National Tuberculosis Reference Laboratory (NTRL), *Centre Pasteur du Cameroun* (CPC), which is an accredited laboratory to ISO 15189 and demonstrates proficiency through participation in DST proficiency testing with a TB supranational laboratory (Institute of Tropical medicine, Antwerp). A purposive sampling method was employed to select a total of 500 MTB clinical isolates that were resistant to INH. These isolates were retrieved from a collection of samples stored in the mycobacteriology unit of CPC, specifically those collected between January 1st, 2014, and December 31st, 2020. Phenotypic DST of the isolates was performed by laboratory technicians during the collection period, following the manufacturer's protocol for the BACTEC MGIT 960 system. In the current study, repeat phenotypic DST was conducted only on isolates with discrepancies in the genotypic DST results to validate the susceptibility patterns. Phenotypic DST data, along with patient demographic and clinical variables (sex, age, and sample type), were retrieved from reports stored in the NTRL registers and the CPC database, with the selection of demographic and clinical variables primarily determined by the availability of data. Since the samples were obtained from the NTRL, which analyzes specimens from presumptive TB patients across approximately 93 Diagnosis and Treatment Centers in the Centre, East, and South Regions of Cameroon [20], the investigated isolates are representative of the entire country.

Study population

The study population included MTB clinical isolates previously characterized to be phenotypically resistant to INH with or without concomitant resistance to other first-line anti-TB drugs that were found in the Mycobacteriology repository of the CPC during the study period. Isolates with unknown patient demographics (age, sex and sample type) were excluded from the study.

Laboratory testing

Mycobacterium tuberculosis isolates collection

A total of 500 INH-R MTB clinical isolates were obtained from the mycobacteriology repository. The data of the isolates retrieved were extracted from the TB-NRL registers and the CPC database, and 22 isolates were eliminated from the study due to the absence of data regarding the age, sex and sample type. The sources of origin of the remaining clinical specimens included pulmonary (432 isolates) and extrapulmonary (46 isolates) TB samples. The isolates included 91 INH-monoresistant, 331 MDR (resistant to RIF, with or without resistance to other first-line anti-TB drugs), and 56 polyresistant (resistant to both INH and STR) TB isolates.

Bacterial culture

MTB culturing and manipulation were conducted in a Class II biosafety cabinet within the BSL-3 Mycobacteriology Laboratory at CPC. The bacterial culture was conducted using an automated BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 system (Becton Dickinson International BV, Erembodegem-Belgium) according to the manufacturer's instructions. A 500 µl (µL) aliquot of the retrieved sample was inoculated in MGIT supplemented with 800 µL of reconstituted growth supplement and a mixture of antibiotics consisting of polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin (PANTA) (Becton, Dickinson and Company in Sparks, MD, USA). The inoculated MGIT was then placed in the BACTEC MGIT 960 instrument until it flagged positive or for a maximum of 42 days. A culture was reported to be negative only if there was no growth after 42 days of incubation. All MGIT culture tubes that were positive were removed from the MGIT 960 system, confirmed for acid-fast bacilli (AFB) by Ziehl–Neelsen staining and subjected to identification of the MTB complex using a rapid immunochromatographic test (TB Ag MPT64 test, SD Bioline, Suwon, Korea). The purity of the MGIT culture tubes containing the MTB complex was also checked for contamination with other bacteria or fungi if their subculture grew on blood agar media at 37 °C after 24 h.

Genotypic DST using the line probe assay

Positive MTB isolates were subjected to GenoType MTBDR*plus* testing to identify *katG* and *inhA* mutations. GenoType MTBDR*plus* assay version 2.0 (Hain Lifescience, Nehren, Germany) was performed according to the manufacturer's protocol. The assay is a deoxyribonucleic acid (DNA) strip-based technology comprised of 3 major steps: DNA extraction, multiplex polymerase chain reaction (PCR) amplification and reverse hybridization. These procedures were conducted in three distinct rooms. DNA extraction was conducted in a class II biosafety cabinet, where DNA was isolated from pure positive MGIT cultures using the GenoLyse® Kit (Hain Lifescience, Germany) adhering to the manufacturer's instructions. Five µL of the extracted DNA was used for PCR. PCR amplification was carried out using biotin-labelled

primers under the following conditions: 15 min of denaturation at 95 °C; 10 cycles of 30 s at 95 °C and 120 s at 58 °C; 20 additional cycles of 25 s at 95 °C, 40 s at 53 °C, and 40 s at 70 °C; and a final extension at 70 °C for 8 min. For the hybridization process, 20 µL of the amplicon was mixed with 20 µL of the denaturing reagent (provided in the kit) and the mixture was incubated at room temperature for five minutes. Following this, hybridization buffer was added, followed by stringent buffer and conjugate treatment. The streptavidin-conjugated alkaline phosphatase binds to the amplicon and visible bands appeared following the addition of the substrate. The strips were then washed, removed, and affixed to the assay worksheet for interpretation. Drug resistance was expressed as i) the absence of one or more wild type (WT) bands, ii) the presence of mutant bands with or iii) without the simultaneous absence of the complementary WT. The simultaneous presence of WT and corresponding mutant bands was referred to as a mixed pattern (heteroresistant). An MTB H37Rv ATCC 25177 and molecular grade water were used as positive and negative quality controls respectively for every test.

Phenotypic DST

MTB strains without mutations in the *katG* 315 codon and the *inhA* promoter region were subjected to DST for INH on the BACTEC MGIT 960 platform to validate the susceptibility patterns to INH. This was done following standard manufacturer protocol and WHO critical concentration (CC) of 0.1 mg/L for INH. Bacteria inoculum was prepared from positive MGIT tubes within 5 days of positivity. Following positivity (Day 0), tubes that flagged positive on days 1 or 2, were directly inoculated while tubes that flagged positive on days 3 to 5, were diluted (1:5) with sterile saline. Briefly, lyophilized preparations of the INH drug were reconstituted in sterile distilled water to obtain a drug stock solution with a concentration of 8.0 µg/mL. Two tubes per isolate were labelled: one as a growth control (GC) and the other as an INH drug-containing tube. In each tube, a 0.8 mL BACTEC MGIT SIRE supplement was added. The GC tube remained drug-free, while the corresponding INH tube received 0.1 mL of the drug suspension using sterile pipette tips. Additionally, 0.5 mL of bacterial suspension was inoculated into the drug-containing tube while the GC tube received 0.5 mL of a 1:100 dilution of the bacterial suspension. The tubes were incubated in the BACTEC MGIT 960 instrument until they flagged positive. The MGIT 960 system's software algorithm analyzed the fluorescence of the drug-containing tubes relative to the GC tube to determine antibiotic susceptibility results. The growth ratio between the drug-containing tubes and the growth tube was assessed by the system's software algorithm within 4–13 days once the GC tube reached 400 growth units. The final interpretation and the susceptibility results were reported by the MGIT 960 instrument automatically.

Data analysis

The data were initially input into Microsoft Excel 365 (Version 2402 Build 16.0.17328.20124), and frequencies were utilized to characterize clinical and sociodemographic traits, as well as mutations associated with drug resistance. The prevalence of mutations in the *katG* and *inhA* genes among different sex, age groups, and sample types was compared using the Chi-square test (Fisher's exact test was used when the expected number was less than one). Statistical analysis was conducted using GraphPad Prism Updater, version 10.0.3 software (©1992-2023 GraphPad Software. LLC). A p-value of < 0.05 was considered to indicate statistical significance.

Results

Demographic and clinical characteristics of culture-positive MTB isolates

Out of the 478 study isolates, 410 (85.8%) tested positive on culture and AFB smear (Fig. 1). The sources of origin of the clinical specimens included pulmonary (432 isolates) and extrapulmonary (46 isolates) TB samples. Phenotypically, 286 were MDR-TB isolates; that is resistant to INH and RIF (of which 212 were resistant to 3 or more first-line anti-TB drugs) and 124 isolates were INH-monoresistant TB isolates with or without resistance to other first-line anti-TB drugs (of which 80 were resistant to INH alone and 44 were resistant to both INH and STR). All positive cultures underwent testing with the GenoType MTBDR*plus* assay. Considering the phenotypic DST as the gold standard, the GenoType MTBDR*plus* test correctly identified INH resistance in 263 of 286 MDR-TB isolates (92.0%) and 91 of 124 INH-monoresistant TB isolates (73.4%). The assay revealed a discrepancy in 56 isolates of which 23 (8.0%) were MDR-TB cases and 33 (26.6%) were INH-monoresistant TB cases. Details of the resistance phenotypes and mutation profile of these positive isolates are available in Supplementary Table S1.

Table 1 presents the distribution of sex among patients, with 228/410 (55.6%) males and 182/410 (44.4%) females. Among males, 78/228 (34.2%) cases were identified as resistant to INH, with or without resistance to other first-line anti-TB drugs (henceforth referred to as INH-monoresistant) compared to 46/182 (25.3%) females. A higher proportion of MDR-TB cases was reported among females (136/182, 74.7%) compared to males (150/228, 65.8%), though this difference was not statistically significant ($p = 0.0523$). The average age of the patients was

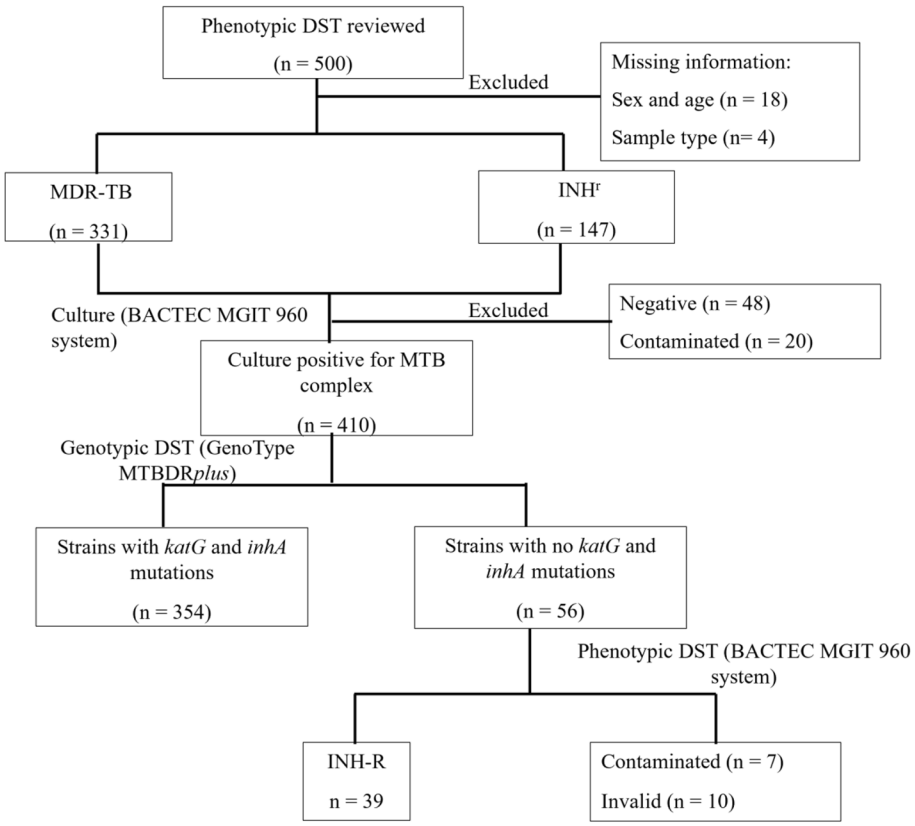

Fig. 1 Flow diagram of the study workflow and drug resistance patterns in the MTB isolates. Key: MDR-TB: isolates resistant to at least two of the first-line drugs INH and RIF; INH^r: INH-monoresistant isolates with or without resistance to other first-line anti-TB drugs; INH-R: isoniazid-resistant

Table 1 Association of phenotypic resistance patterns with the demographic and clinical variables of patients

Parameter	Total	INH^r		MDR-TB		*p*-value
		No. of isolates	%	No. of isolates	%	
Sex						
Male	228	78	34.21	150	65.79	0.0523*
Female	182	46	25.27	136	74.73	
Age (years)						
< 15	6	2	33.33	4	66.67	0.0016*
15–29	138	31	22.46	107	77.54	
30–44	170	51	30.00	119	70.00	
45–59	71	24	33.80	47	66.20	
≥ 60	25	16	64.00	9	36.00	
Sample type						
Pulmonary TB	371	97	26.15	274	73.85	< 0.0001*
Extrapulmonary TB	39	27	69.23	12	30.77	

Key: *No.* Number, *MDR-TB* Isolates resistant to at least two of the first-line drugs INH and RIF, *INH^r* INH monoresistant isolates with or without resistance to other first-line anti-TB drugs

* *p*-value from Fisher's exact test

36.3 ± 13.4 years. The age distribution revealed a statistically significant difference in the resistance profiles across different age groups ($p = 0.0016$). Notably, the highest percentage of MDR-TB cases is observed in the economically active group of ages between 15–59 years. Regarding TB sample types, a highly significant difference was observed between the sample types ($p < 0.0001$), with pulmonary TB samples showing a higher prevalence of MDR-TB isolates (274/371, 73.9%) compared to extrapulmonary TB samples (12/39, 30.8%) (Table 1).

Frequencies and patterns of *katG* and *inhA* mutations associated with INH resistance

Of the phenotypically INH-R isolates, 354/410 (86.3%) isolates possessed INH resistance-conferring mutations at either *katG* codon 315 or in the *fabG1-inhA* regulatory region while 56/410 (13.7%) isolates had no mutations at either locus. The primary genetic mutation responsible for INH resistance was observed in the *katG* gene in 247/410 (60.2%) isolates (Table 2). Among these, 61/247 (24.7%) were INH-monoresistant isolates and 186/247 (75.3%) were MDR-TB isolates. Specifically, the *katG* S315T1 mutation was the predominant *katG* mutation identified in this study, characterized by the absence of the WT band and the presence of a corresponding mutant (MUT1) band. This mutation was observed in 213/410 (52%) INH-R isolates, with 51/213 (23.9%) present in INH-monoresistant and 162/213 (76.1%) in MDR-TB isolates. Additionally, 5/410 (1.2%) isolates showed a missing WT band without the corresponding MUT band, all of which were MDR-TB cases. Furthermore, among the *katG* mutants, 29/410 (7.1%) of the isolates showed simultaneous hybridization of the WT and MUT bands, referred to as heteroresistant, with 10/29 (34.5%) occurring in INH-monoresistant isolates and 19/29 (65.5%) in MDR-TB isolates (Table 2).

Mutations in the *inhA* promoter region were detected in 76/410 (18.5%) INH-R isolates, with 29/76 (38.2%) occurring in INH-monoresistant and 47 (61.8%) in MDR-TB isolates (Table 2). The most prevalent *inhA* promoter mutation identified by the absence of the WT1 band and the presence of the MUT1 band (C-15T) was observed in 59/410 (14.4%) isolates. Of these mutations, 25/59 (42.4%) were found in INH-monoresistant isolates, and 34/59 (57.6%) in MDR-TB isolates. Following C-15T, the T-8A mutation, indicated by the absence of the WT2 band and the presence of the MUT3B band was identified in 5/410 (1.2%). Additionally, heteroresistance at the *inhA* promoter region was observed in 12/410 (2.9%) isolates, with 2 (16.7%) in INH-monoresistant isolates and 10 (83.3%) in MDR-TB isolates.

Table 2 Mutation patterns of INH-R MTB clinical isolates detected by the GenoType MTBDR*plus* assay

Gene	Band missing	Gene region	Mutation present	INH^r No. (%)	MDR-TB	Total
katG	WT	315	S315T1	51 (23.94)	162 (76.06)	213 (51.95)
	WT	315	Uk	0 (0.00)	5 (100.00)	5 (1.22)
		HR	WT & S315T1	10 (34.48)	19 (65.52)	29 (7.07)
	Total			**61 (24.70)**	**186 (75.30)**	**247 (60.24)**
inhA	WT1	-15/-16	C-15T	25 (42.37)	34 (57.63)	59 (14.39)
	WT2	-8	T-8A	2 (40.00)	3 (60.00)	5 (1.22)
		HR	WT1 & C-15T	2 (22.22)	7 (77.78)	9 (2.20)
			WT2 & T-8A	0	2 (100.00)	2 (0.49)
			WT1, WT2, C-15T & T-8C	0	1 (100.00)	1 (0.24)
	Total			**29 (38.16)**	**47 (61.84)**	**76 (18.54)**
katG & *inhA*	WT & WT1	315 & -15/-16	S315T1 & C-15T	0	5 (100.00)	5 (1.22)
	WT & WT2	315 & -8	S315T1 & T-8C	0	15 (100.00)	15 (3.66)
	WT & WT2	315 & -8	S315T1 & T-8A	0	1 (100.00)	1 (0.24)
		HR	WT, S315T1 & WT1, C-15T	0	7 (100.00)	7 (1.71)
			WT, S315T1 & WT2, T-8C	1 (50.00)	1 (50.00)	2 (0.49)
			WT, S315T1 & WT1, WT2, C-15T, T-8C	0	1 (100.00)	1 (0.24)
	Total			**1 (3.23)**	**30 (96.77)**	**31 (7.56)**
			No mutation in *katG* & *inhA*	**33 (58.93)**	**23 (41.07)**	**56 (13.66)**
Overall total				**91 (22.20)**	**263 (64.15)**	**410**

Key: *WT* Wild type, *HR* Heteroresistant, *Uk* Unknown, *No.* Number, *MDR-TB* Isolates resistant to at least two of the first-line drugs INH and RIF, *INH^r* INH monoresistant isolates with or without resistance to other first-line anti-TB drugs

Co-mutations of *katG* and *inhA* were present in 31/410 (7.6%) INH-R isolates, with 1/31 (3.2%) in INH-monoresistant and 30/31 (96.8%) in MDR-TB isolates (Table 2). The most common co-mutation was the S315T1/T-8C mutation, seen only in MDR-TB isolates (15/31, 48.4%). Heteroresistant isolates with co-mutations were identified in 10/31 (32.3%) isolates.

As shown in Table 3, of the 410 INH-R isolates, the proportion of *katG* codon 315 and *inhA* promoter mutations significantly differed between INH-monoresistant and MDR-TB isolates ($p=0.0004$). The *katG* mutations are predominantly found in MDR-TB isolates (186/247, 75.3%) while *inhA* mutations show a lower prevalence of MDR-TB cases (47/76, 61.8%).

Factors associated with *katG* S315T1 and *inhA* promoter mutations in INH-R MTB isolates

We further explored genetic mutation profiles concerning patient demographics (sex, age, and sample type) among the INH-R isolates. As detailed in Table 4, the *katG* S315T1 mutation alone was most observed, accounting for 141/228 (61.8%) in males and 106/182 (58.2%) in females, while mutations in the *inhA* promoter region (C-15 T, T-8C, and T-8A) alone were observed in 44/228 (19.3%) males and 32/182 (17.6%) females. In addition, dual *katG* and *inhA* mutations were observed in both males and females; 8/228 (3.5%) and 23 (12.6%) respectively. A statistically significant difference ($p=0.0056$) was observed with a higher percentage of males exhibiting *katG* mutations compared to females. Age was significantly associated with mutations in the *katG* gene and the *inhA* promoter region ($p=0.0004$). The age distribution showed significant differences in mutation patterns, particularly within the economically active group (ages between 15-59 years), with *katG* mutation predominating in all ($p=0.0004$). Furthermore, the presence of *katG*, *inhA* and dual *katG* and *inhA* mutations were more common in pulmonary TB individuals 229/371 (61.7%),

Table 3 Association of *katG* codon 315 and *inhA* promoter mutations in INH-R MTB isolates

Gene mutation	INH^r		MDR-TB		Total n (%)	*p*-value
	No.	%	No.	%		
katG mutations	61	24.70	186	75.30	247 (60.24)	0.0004*
inhA promoter mutations	29	38.16	47	61.84	76 (18.54)	
katG & *inhA* co-mutations	1	3.23	30	96.77	31 (7.56)	
Total	91	25.71	263	74.29	410	

Key: *No.* Number, *MDR-TB* Isolates resistant to at least two of the first-line drugs INH and RIF, *INH^r* INH monoresistant isolates with or without resistance to other first-line anti-TB drugs

* *p*-value from Fisher's exact test

Table 4 Associations with patient characteristics for *katG* codon 315 and *inhA* promoter mutations in INH-R MTB isolates

Parameter	No. of patients	*katG*[a]	*inhA*[b]	*katG* & *inhA*	*p*-value
Total	410	247 (60.24)	76 (18.54)	31 (7.56)	
Sex					
Male	228	141 (61.84)	44 (19.30)	8 (3.51)	0.0056*
Female	182	106 (58.24)	32 (17.58)	23 (12.64)	
Age group (years)					
<15	6	3 (50.00)	3 (50.00)	0 (0.00)	0.0004
15-29	138	83 (60.14)	30 (21.74)	11 (7.97)	
30-44	170	104 (61.18)	20 (11.76)	18 (10.59)	
45-59	71	48 (67.61)	13 (18.31)	2 (2.82)	
≥60	25	9 (36.00)	13 (52.00)	0 (0.00)	
Sample type					
Pulmonary TB	371	229 (61.73)	68 (18.33)	30 (8.09)	0.0127*
Extrapulmonary TB	39	18 (7.53)	8 (3.35)	1 (0.42)	

Key: *No.* Number

[a] without *inhA* mutation

[b] without *katG* mutation

* *p*-value from Fisher's exact test

68/371 (18.3%) and 30/371 (8.1%) respectively) compared to those with extrapulmonary TB. This difference was statistically significant ($p=0.0127$) (Table 4).

This study further examined separately the associations of *inhA* promoter mutations with patient demographics in INH-R isolates. Table 5 depicts that sex and sample type were not linked to *inhA* mutations in INH-R isolates. Though slightly higher percentages of *inhA* mutations were reported in males (22.8%) compared to females (19.9%), likewise in extrapulmonary samples (29.6%) compared to pulmonary samples (27.5%), the differences were not statistically significant. However, a highly significant association between age and the presence of *inhA* mutation was observed, with the highest proportion found within the economically active population (15-59 years) ($p=<0.0001$).

Following the same trend, as indicated in Table 6, sex did not show an association with *katG* mutations in INH-R isolates. The percentage of *katG* mutations reported in females (85/182, 46.7%) was slightly higher than in males (101/228, 44.3%), though this difference was not statistically significant. Likewise, there was no

Table 5 Associations with patient characteristics for *inhA* promoter mutations in INH-R MTB isolates

Parameter	No. of patients	INH^r		MDR-TB		*p*-value
		No.	%	No.	%	
Sex						
Male	228	20	8.77	24	10.53	0.3041*
Female	182	9	4.95	23	12.64	
Age group (years)						
< 15	6	0	0.00	0	0.00	< 0.0001
15–29	138	6	4.35	24	17.39	
30–44	170	8	4.71	12	7.06	
45–59	71	5	7.04	8	11.27	
≥ 60	25	10	40.00	3	12.00	
Sample type						
Pulmonary TB	371	21	5.66	47	12.67	0.0004*
Extrapulmonary TB	239	8	3.35	0	0.00	

Key: *MDR-TB* Isolates resistant to at least two of the first-line drugs INH and RIF, *INH^r* INH-monoresistant isolates with or without resistance to other first-line anti-TB drugs

* *p*-value from Fisher's exact test

Table 6 Associations with patient characteristics for *katG* codon 315 mutations in INH-R MTB isolates

Parameter	No. of patients	INH^r		MDR-TB		*p*-value
		No.	%	No.	%	
Sex						
Male	228	40	17.54	101	44.30	0.2385*
Female	182	21	11.54	85	46.70	
Age group (years)						
< 15	6	0	0.00	3	50.00	0.0612
15–29	138	16	11.59	67	48.55	
30–44	170	28	16.47	76	44.71	
45–59	71	13	18.31	35	49.30	
≥ 60	25	4	16.00	5	20.00	
Sample type						
Pulmonary TB	371	52	14.02	177	47.71	0.0095*
Extrapulmonary TB	39	9	23.08	9	23.08	

Key: *No.* Number, *MDR-TB* Isolates resistant to at least two of the first-line drugs INH and RIF, *INH^r* INH-monoresistant isolates with or without resistance to other first-line anti-TB drugs

* *p*-value from Fisher's exact test

statistically significant difference in the prevalence of *katG* mutations across different age groups ($p=0.0612$), but the highest percentage of this mutation was observed in the < 15 age group (3/6, 50.0%). However, this mutation was prevalent in pulmonary TB isolates (177/371, 47.7%, $p=0.0095$).

Furthermore, it was observed that the disparity in the occurrence of dual *katG* and *inhA* mutations between female individuals with INH-R TB was more significant than in males ($p=0.0007$) (Table 7). Additionally, this mutation was predominantly observed in individuals aged 30-44 years and those with pulmonary TB. However, there was no statistically significant difference in the rates of isolates containing *katG* and *inhA* co-mutations based on age group ($p=0.2091$) or sample type ($p=0.4025$) in INH-R (Table 7).

Discussion

The overall prevalence of resistance to INH globally, either as a standalone drug or in conjunction with other medications, has surpassed 13%, indicating that approximately one in seven TB cases is resistant to INH [9]. The identification of gene mutations in drug-resistant strains may aid in the prediction of the degree of INH resistance and assist clinicians in choosing the best treatment for their patients [12]. This study examined the prevalence of the most common mutations in the *katG* gene and *inhA* promoter region in INH-R MTB clinical isolates which are critical markers of INH resistance in MTB, along with their correlation with patients' demographic details (sex, age, and sample type).

In this study, 86.3% of the phenotypically resistant INH MTB isolates analyzed harboured mutations in the *katG* codon 315 and *inhA* promoter region, underscoring their role in INH resistance. Specifically, mutations in the *katG* gene, essential for INH activation lead to the partial or complete loss of catalase/peroxidase activity, rendering INH ineffective [6, 21]. Patients with these mutations are unlikely to benefit from high-dose INH in modified MDR-TB regimens [7]. Among the isolates from Cameroon, 60.2% had *katG* codon 315 mutations, predominantly the S315T1 variant (52.0%), which was significantly more common in MDR-TB cases (75.3%) compared to INH-monoresistant cases (24.7%) ($p=0.0004$). This supports the hypothesis that the *katG* S315T mutation may precede RIF resistance [22]. The association of *katG* S315T mutations with the MDR-TB phenotype can be attributed to their low fitness cost and the survival advantage they provide to INH-R [9]. Our results align with findings from Iran (53.3%) [22] and higher frequencies reported in studies from South Africa (63.9%) [23], China (63%) [11], Australia (65.4%) [18], India (71%) [21], Uganda (76%) [19] and Ghana (84.3%) [14]. Varying prevalence of *katG* mutation can be due to factors like clinical practices, population genetics and specific MTB strains present [11], and environmental and socio-economic factors that may affect TB transmission dynamics [22]. Interestingly, we identified 1.2% of isolates with unknown *katG* mutation, suggesting they may represent rare variants not detected by rapid molecular assays [24]. Notably, no *katG* S315T2 mutations were observed differing from other studies [7, 21, 24].

Table 7 Associations with patient characteristics for *katG* codon 315 and *inhA* promoter co-mutations in INH-R MTB isolates

Parameter	No. of patients	INH^r		MDR-TB		*p*-value
		No.	%	No.	%	
Sex						
Male	228	0	0.00	8	3.51	0.0007*
Female	182	1	0.55	22	12.09	
Age group (years)						
< 15	6	0	0.00	0	0.00	0.2091*
15–29	138	1	0.72	10	7.25	
30–44	170	0	0.00	18	13.04	
45–59	71	0	0.00	2	2.82	
≥ 60	25	0	0.00	0	0.00	
Sample type						
Pulmonary TB	371	1	0.27	29	7.82	0.4025*
Extrapulmonary TB	39	0	0.00	1	2.56	

Key: *No.* Number, *MDR-TB* Isolates resistant to at least two of the first-line drugs INH and RIF, *INH^r* INH-monoresistant isolates with or without resistance to other first-line anti-TB drugs

* *p*-value from Fisher's exact test

Mutations in the *inhA* promoter region are the second most common cause of INH resistance, facilitating low-level resistance through the overexpression of the drug target, enoyl acyl carrier reductase [9, 11, 22, 25]. Individuals with low-level INH resistance due to *inhA* mutations may benefit from high doses of INH (10–15 mg/kg/day) [26]. Our study revealed that 18.5% of cases had *inhA* promoter mutations, with a frequency of 38.2% in INH-monoresistant isolates and 61.8% in MDR-TB isolates. The predominant mutation, C-15T was present in 14.4% of all INH-R isolates. Comparatively, the prevalence of *inhA* mutations varies widely across different regions, ranging from 5.4% to 71.9% [10, 14, 19, 21, 23, 27, 28]. This variation may reflect the diverse molecular characteristics of predominant MTB strains in different geographic areas [6]. Additionally, other mutations in the *inhA* promoter region, such as T-8A (1.2%), occur at much lower frequencies compared to C-15T mutation. This aligns with findings from other investigators [19, 29] and underscores the need for region-specific assessments of *inhA* mutations to inform treatment strategies.

The presence of both *katG* and *inhA* mutations indicates a high degree of INH resistance, making higher doses of INH unlikely to enhance treatment efficacy [26]. This study revealed a 7.6% prevalence of *katG* and *inhA* co-mutation among the tested isolates, which compares to those obtained in India (6.0%) [26], Poland (11.1%) [9], and China (1.1%) [11], while some studies reported no instances [21].

Heteroresistance represents a critical initial stage in the transition from drug-susceptible to mono-resistant and/or MDR-TB isolates [15]. This phenomenon poses a challenge in TB diagnosis and has been linked with poor treatment outcomes and subsequent drug-resistant TB development [30]. Our findings demonstrated a 12.4% prevalence of heteroresistance among the analyzed INH-R isolates, with 3.2% in INH-monoresistant isolates and 9.2% in MDR-TB isolates. Heteroresistance may result from the transmission of both resistant and susceptible bacterial populations or the gradual transition of sensitive bacteria at disease onset to drug-resistant bacteria during therapy [31]. The high prevalence of heteroresistance in our study suggests mixed infections which can accelerate treatment failure, promote the transmission of drug-resistant MTB strains, and facilitate the acquisition of mutations [27]. Additionally, heteroresistance has significant implications for TB diagnosis. However, the phenotypic DST and rapid molecular assays are limited and may miss heteroresistant populations due to their limited sensitivity to detect minor resistant subpopulations, particularly at low frequencies. This diagnostic gap may result in the misclassification of heteroresistant cases as fully susceptible, leading to inappropriate treatment regimens and delayed interventions. Comparative examination reveals significant variations in heteroresistance prevalence across studies: 57.5% in South Africa [27], 11.2% in India [7] and 5% in a systematic review [15]. These discrepancies may stem from differences in sample sizes, detection methodologies [15] and the underlying TB epidemiology and healthcare systems in various regions.

Drug-resistant TB can spread when it is undetected and treated inappropriately [29]. Notably, 10%–25% of INH-R isolates exhibit mutations outside the *katG* and *inhA* loci, exhibiting unclear alterations [32]. Our analysis found that 13.7% of the INH-R isolates analyzed lacked mutations in the *katG* codon 315 and *inhA* promoter region. These unknown resistance mechanisms may be explained following a significant drawback of the current rapid molecular test [22] and the potential confounding effects of heteroresistance from mixed strains [19]. Such scenarios increase the number of undetected isolates progressing into MDR-TB due to inadequate treatment. Therefore, more discriminatory techniques are necessary for better analysis of these isolates.

Our analysis found no significant association between INH resistance and sex ($p=0.0523$), contrasting with other studies suggesting males are more affected by drug-resistant strains [33]. This discrepancy may result from differences in population characteristics, study design, or unmeasured confounders. Additionally, behavioural or biological differences influencing drug resistance might vary across populations. However, a significant association was found between mutation prevalence and sex, with males showing a higher percentage of INH resistance-conferring mutations ($p=0.0056$), aligning with literature attributing this to greater exposure factors [21, 34], immune differences, and socioeconomic. Furthermore, the observed disparity in dual *katG* and *inhA* mutations between female and male individuals with INH-R TB ($p=0.0007$), despite no association found with sex for *katG*-only and *inhA*-only mutations, may warrant further investigations as this suggests that the mechanisms behind dual mutations could be distinct from those causing single mutations.

Age significantly influenced resistance and mutation prevalence, with the highest rates in the economically active group (ages between 15-59 years, $p=0.0004$), consistent with increased social mobility and exposure to untreated cases [33].

Pulmonary TB samples exhibited higher mutation prevalence than extrapulmonary TB samples (88.1% vs. 69.2%, $p=0.0127$), likely reflecting bacterial load, infectivity, and the predominance of pulmonary TB cases [35]. Notably, variability in the association between individual resistance-conferring mutations and patient parameters

likely arises from differences in mutation biology, treatment regimens, transmission dynamics and sample-specific factors influencing mutation selection.

This study identifies several areas for future research. To improve our findings, it is essential to incorporate a broader range of samples from other regions, as the TB-NRL currently receives samples from only 3 out of 10 regions in Cameroon. The retrospective design may have introduced selection or information bias. Additionally, the lack of investigation into second-line drug resistance means some isolates might have been misclassified as MDR-TB instead of pre-extensively drug-resistant (Pre-XDR) TB. We also lack data on key clinical and socio-demographic risk factors, such as alcoholism, smoking, and comorbidities like diabetes and HIV. Lastly, relying solely on the Genotype MTBDR*plus* assay limits our understanding of resistance mechanisms. Therefore, utilization of a high-throughput technique such as next-generation sequencing to explore these resistance mechanisms, investigate genomic epidemiology of the strains, detect heteroresistance and assess comprehensively the associated risk factors can inform public health intervention.

Conclusion

The findings of this study confirmed the prevalence of the *katG* S315T1 substitution as a reliable indicator of INH resistance, with the *inhA* C-15T mutation providing additional support. Our results highlight a significant correlation between patient age, sex and sample type with the presence of common mutations in the *katG* gene and *inhA* promoter region, highlighting the importance of demographic factors in understanding these mutations. Given that these mutations are strongly associated with varying levels of INH resistance, it is crucial that TB management strategies incorporate genetic profiling alongside patient demographics to optimize treatment outcomes and enhance control measures. Notably, a substantial proportion of INH-R isolates exhibited no evidence of the common *katG* S315T1 and *inhA* promoter mutations, underscoring the urgent need for improved molecular detection methods to identify a wider array of resistance mechanisms. Additionally, our findings emphasize the importance of understanding heteroresistance in diverse settings, as it can inform targeted interventions, improve diagnostic strategies and optimize treatment outcomes to combat the rising threat of drug-resistant TB.

Abbreviations

COVID-19	Coronavirus disease
CPC	*Centre Pasteur du Cameroun*
DNA	Deoxyribonucleic acid
DST	Drug susceptibility testing
GC	Growth control
HIV	Human Immunodeficiency Virus
HR	Heteroresistance
INH	Isoniazid
INH^r	INH-monoresistant isolates with or without resistance to other first-line anti-TB drugs
INH-R	Isoniazid-resistant
LPA	Line Probe Assay
MDR-TB	Multidrug-resistant tuberculosis
MGIT	Mycobacterial Growth Indicator Tube
MTB	*Mycobacterium tuberculosis*
MUT	Mutation
No.	Number
NTRL	National Tuberculosis Reference Laboratory
OADC	Oleic Albumin Dextrose Catalase
PANTA	Polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin
PCR	Polymerase chain reaction
RIF	Rifampicin
RR-TB	Rifampicin-resistant tuberculosis
STR	Streptomycin
TB	Tuberculosis
WHO	World Health Organization
WT	Wild type

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

V.F.D.D conceived and designed the intellectual content; S.N.T, Y.W.K.S, B.S.T, Y.J.D, Y.P.A, and S.N.A. screened the NTRL information system for the isolates that were used in the project; V.N.N conducted the laboratory analysis; V.N.N, N.A.M, and E.M. analyzed and interpreted the data; E.A.N, V.F.D.D, and A.M. supervised the laboratory work; S.E coordinated research activities; V.N.N. wrote the manuscript draft. All the authors reviewed and edited the draft. All the authors have read and approved the final manuscript and agreed to be responsible for all aspects of the work.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was ethically approved by the Centre Regional Ethics Committee for Human Health Research in Cameroon (CRERSH-Ce) under approval number 00952/CRERSHC/2022.

The study does not include any personal data. It utilized archived MTB clinical isolates routinely obtained from patients for diagnostic and therapeutic purposes.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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