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Prevalence of drug resistance-conferring mutations associated with isoniazid- and rifampicin-resistant Mycobacterium tuberculosis in Ethiopia: a systematic review and meta-analysis

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ABSTRACT

Objectives: Globally, the incidence and mortality of tuberculosis (TB) are declining; however, low detection of drug-resistant disease threatens to reverse current progress toward global TB control. Multiple rapid molecular diagnostic tests have recently been developed to detect genetic mutations in *Mycobacterium tuberculosis* (Mtb) known to confer drug resistance. However, their utility depends on the frequency and distribution of resistance-associated mutations in the pathogen population. This review aimed to assess the prevalence of gene mutations associated with rifampicin (RIF)- and isoniazid (INH)-resistant Mtb in Ethiopia.

Methods: We searched the literature in PubMed/MEDLINE, Web of Science, Scopus and Cochrane Library. Data analysis was conducted in Stata 11.

Results: Totally, 909 (95.8%) of 949 INH-resistant Mtb isolates had detectable gene mutations: 95.8% in *katG*315 and 5.9% in the *inhA* promoter region. Meta-analysis resulted in an estimated pooled prevalence of *katG*MUT1(S315T1) of 89.2% (95% CI 81.94–96.43%) and a pooled prevalence of *inhA*MUT1(C15T) of 77.5% (95% CI 57.84–97.13%). Moreover, 769 (90.8%) of 847 RIF-resistant strains had detectable *rpoB* gene mutations. Meta-analysis resulted in a pooled prevalence of *rpoB*MUT3(S531L) of 74.2% (95% CI 66.39–82.00%).

Conclusion: RIF-resistant Mtb were widespread, particularly those harbouring *rpoB*(S531L) mutation. Similarly, INH-resistant Mtb with *katG*(S315T1) and *inhA*(C15T) mutations were common. Tracking S531L, S315T1 and C15T mutations among RIF- and INH-resistant isolates, respectively, would be diagnostically and epidemiologically valuable. Rapid diagnosis of RIF- and INH-resistant Mtb would expedite modification of TB treatment regimens, and proper timely infection control interventions could reduce the risk of development and transmission of multidrug-resistant TB.

Introduction

Tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis* (Mtb) bacilli, remains a major global health threat [1–3]. It is the leading cause of death from a single infectious agent, ranking above human immunodeficiency virus/acquired immunodeficiency syndrome (HIV)/AIDS [1,3]. Globally, despite the decrease in TB incidence and mortality over the past decades, there were still an estimated 10 million people infected with TB and approximately 1.41 million deaths attributed to TB in 2019 [3]. TB affects all countries and all age groups, but the epidemiological distribution of TB cases is heavily skewed towards low-income countries [1,3]. Pandemics of HIV/AIDS, deterioration of public-health systems and the emergence of multidrug-resistant (MDR) forms of TB have worsened the spread of TB in developing countries, including Ethiopia [4].

According to the World Health Organization (WHO) [3], in 2019 Ethiopia stood 12th in the world and 4th in Africa among the high TB burden countries, with 23 800 TB deaths and 157 000 new TB cases. In Ethiopia, in 2019 the prevalence of MDR-TB, defined as

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resistance to both rifampicin (RIF) and isoniazid (INH), the two most important antibiotics for the treatment of TB patients, was 0.71% among newly diagnosed TB cases and 12% among previously treated individuals [3]. As in other low- and middle-income countries, TB/HIV co-infection and the emergence of MDR-TB strains are becoming pressing challenges in the efforts to control TB in Ethiopia [1,5,6]. All recent data show that TB and drug-resistant forms of TB remain a major public-health concern in Ethiopia. Moreover, TB was also the leading cause of hospital deaths in Ethiopia. Some of the primary factors associated with the increased spread of pulmonary TB in Ethiopia are HIV/AIDS, poverty, lack of access to healthcare, weak medical services, overcrowded living conditions, a weak TB control programme, and increased vulnerability of infants and the elderly [7]. The high prevalence of diabetes mellitus, alcoholism and smoking are also significant factors [7].

The emergence of antimycobacterial drug resistance is threatening TB prevention and control activities, and TB remains a major public-health threat on a global scale [2]. Worldwide, in 2019 there were approximately half a million new cases of RIF-resistant TB, of which 78% were MDR-TB [3]. Resistance to anti-TB drugs in Mtb arises as a result of spontaneous gene mutations that reduce the bacterium's susceptibility to the most commonly used anti-TB drugs. These genes can encode drug targets or drug metabolism mechanisms and influence the efficacy of anti-TB treatments [1,2,8–10]. Inappropriate treatment and poor patient adherence to anti-TB drug regimens contribute to the development of drug-resistant TB (DR-TB), while the lack of drug resistance diagnosis and subsequent improper TB treatment increase the risk of direct transmission of DR-TB [8,9,11].

Due to the lack of accurate, rapid and inexpensive diagnostic tests, there is incomplete reporting of drug resistance, including MDR-TB detection rates, in resource-limited countries, including Ethiopia. Sputum smear microscopy is the most commonly used TB diagnostic method, but it has low sensitivity and cannot detect drug resistance [1]. Mycobacterial culture on liquid or solid media and standard drug susceptibility testing (DST) is slow in obtaining results for informed initiation of appropriate anti-TB drug treatment, and it requires well-furnished laboratory settings and substantial biosafety resources. This is impracticable in many low-resource settings, including Ethiopia [1,2,9,12]. Furthermore, phenotypic testing often lacks accuracy and reproducibility [13]. For these reasons, the use of rapid molecular tests is increasing worldwide. Rapid molecular diagnostic assays, such as the Xpert® MTB/RIF and Ultra assays (Cepheid, Sunnyvale, CA, USA), and the GenoType® MTBDR*plus* and MTBDR*sl* line probe assays (Hain Lifescience GmbH, Nehren, Germany), have been shown to reduce the time to treatment initiation in TB and DR-TB patients [8,13,14]. In pursuance of these molecular diagnostic approaches to correctly identify all resistant Mtb isolates, the genes and specific nucleotide changes conferring anti-TB drug resistance should ideally be identified and included in the diagnostic testing protocol. However, the geographical frequency and global distribution of RIF and INH resistance-associated Mtb gene mutations have not yet been thoroughly measured in the pathogen population [15].

Several previous studies have identified different genes that encode anti-TB drug targets and have briefly described different mechanisms of resistance both to RIF and INH [16–21]. More than 95% of RIF resistance is associated with gene mutations in an 81-bp region of the *rpoB* gene. INH resistance appears more complex and has been associated with multiple genes, most commonly *katG* and the promoter region of the *inhA* gene [18,21–24]. Current molecular diagnostic tests for INH resistance rely on detection of the 'canonical' mutations in codon 315 of *katG* and position –15 in the *inhA* promoter region. Many earlier studies have identified highly variable frequencies of these mutations, with *katG*315 mutations accounting for 42–95% and *inhA*–15 mutations accounting for 6–43% of phenotypic INH resistance [8,9,16,20,21,25,26].

To date, there is no systematic review and meta-analysis that has assessed the most common gene mutations conferring RIF and INH resistance in Mtb in Ethiopia. Moreover, the estimated pooled prevalence of RIF resistance-associated gene mutations and the frequencies of co-occurring or multiple mutations have not been evaluated to understand the overall proportion of phenotypic INH and RIF resistance explained by the existing single or canonical gene mutations.

Hence, it is critical to understand the frequency and prevalence of drug resistance-conferring mutations associated with RIF- and INH-resistant Mtb in Ethiopia. Failure to account for these variations limits the local effectiveness of molecular diagnostic tools currently available and constrains the development of improved genotypic diagnostic tests [27]. Therefore, this systematic review and meta-analysis aimed to estimate the frequency and prevalence of the most common gene mutations associated with phenotypic RIF and INH resistance in Mtb in Ethiopia based on previously published literature data.

Methods

2.1. Study protocol

We followed the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) guidelines [28] to search records from online databases, paper screening by title and abstract, and evaluation of the full-text's eligibility (Fig. 1). The completed PRISMA checklist is provided in Supplementary Table S1. This review protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO) (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020186705).

2.2. Databases and search strategy

Articles published in the English language were searched on PubMed/MEDLINE, Web of Science, Scopus and Cochrane Library electronic databases as well as Google Scholar, with no restriction on the year of publication of the study. Studies that reported gene mutations conferring RIF and INH resistance in Mtb in Ethiopia were included in the analysis. We used the following specific subject headings for databases searching: '*Mycobacterium tuberculosis*', 'tuberculosis', 'drug-resistance', 'drug susceptibility testing', 'anti-tuberculosis drug-resistance', 'antitubercular agents', 'first-line antitubercular drugs', 'isoniazid resistance tuberculosis', 'rifampicin resistance tuberculosis', 'gene mutations', 'drug resistance-conferring mutations', 'frequency of gene mutations', 'antitubercular drug-resistance determinants', 'magnitudes of gene mutations', 'molecular diagnostics', 'molecular detection', 'molecular characterization', 'genotyping', 'Line Probe Assay', 'GenoType®MTBDR*plus* assay', 'GeneXpertMTB/RIF assay', 'GenoType®MTBDR*sl* assay' and 'Ethiopia'. The search strings were applied using 'AND' and 'OR' Boolean operators. The PubMed key search terms used were: (*Mycobacterium tuberculosis* [MeSH Terms] OR tuberculosis [MeSH Terms]) AND (INH OR isoniazid [MeSH Terms]) AND (RIF OR rifampicin [MeSH Terms]) AND (resistance OR resistant) AND (mutations [MeSH Terms]) OR sequence) AND Ethiopia. Details of the full search strategy for PubMed/MEDLINE are provided in Supplementary Table S2. Furthermore, we reviewed the reference lists of the primary studies to assess further potential studies and other grey literature.

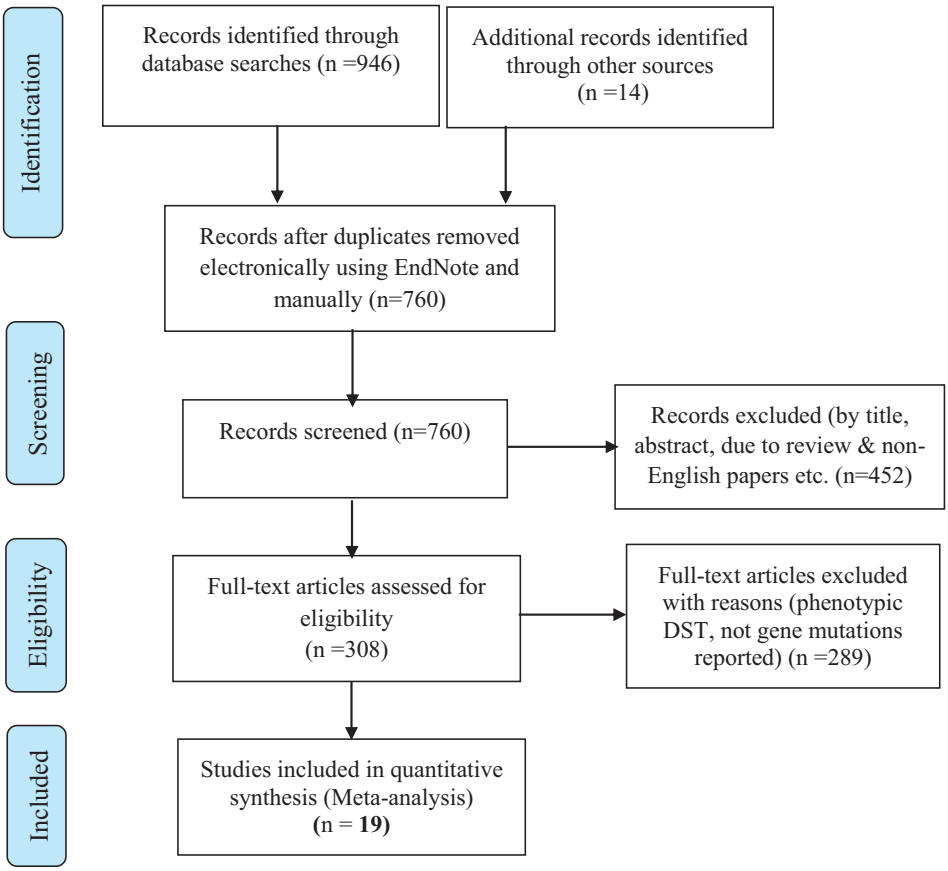

Fig. 1. Flow diagram of the literature search strategy, search results, and reasons for inclusion and exclusion of articles. DST, drug susceptibility testing.

2.3. Screening and eligibility of studies

Papers retrieved from the databases were exported into the reference software EndNote v.8.2 (Thomson Reuters, Stamford, CT, USA). Duplicate papers were sorted, noted and removed using EndNote software. Subsequently, two researchers (MAR and BA) independently evaluated the paper's title and abstracts using the preset inclusion criteria. Two investigators (BA and BBA) also independently collected full-texts and assessed the eligibility of articles to include in the final analysis. In each case, discrepancies that arose between the two authors were resolved through discussion with the other author to come to a consensus.

2.4. Inclusion and exclusion criteria

All observational studies (cross-sectional, case–control and cohort studies) that diagnosed RIF and INH resistance in Mtb using standard WHO-approved molecular DST tools and reported mechanisms of anti-TB drug resistance or the gene mutations conferring RIF and INH resistance in Mtb in Ethiopia were included. Furthermore, studies addressing the frequency of gene mutations and some or all of the following criteria were included: (i) studies reporting data regarding the prevalence of anti-TB drug resistance among pulmonary TB (PTB) and extrapulmonary TB (EPTB) patients (both re-treated and newly diagnosed cases); (ii) the prevalence of any anti-TB drug resistance or MDR-TB or extensively drug-resistant tuberculosis (XDR-TB); (iii) studies that used standard WHO-approved molecular anti-TB DST tools; and (iv) TB research conducted in Ethiopia and published in the English language. We excluded those studies from the analysis with the following exclusion criteria: (i) studies that did not report mechanisms of anti-TB drug resistance or gene mutations conferring RIF and INH resistance in Mtb; (ii) studies reporting data on nontuberculous mycobacteria; (iii) studies that did not assess DST to RIF and INH; and (iv) studies performing anti-TB DST only through phenotypic methods. Editorial reports, narrative reviews, case reports and qualitative studies were excluded from the analysis. Additionally, we excluded citations without full-text after contacting a study author twice by email.

2.5. Quality assessment

The critical quality assessment checklist recommended by the Joanna Briggs Institute was used to evaluate the quality of all included studies [29]. Three investigators (MAR, BA and BBA) independently evaluated the quality of the full-text articles. Discrepancies were resolved through discussion to reach a consensus and to include articles in the final analysis. The domain paper quality assessment criteria were: clear inclusion criteria; details of study subjects and the study settings; reliable/valid measurements for exposure and outcome variables; and appropriate statistical analysis. Studies (case–control, cross-sectional and cohort) with a score of four and above were considered good quality and were included, while studies with an average score of three and below were considered as poor quality and were excluded (Supplementary Table S3).

2.6. Data extraction

We used a standard data extraction format prepared in Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Two authors (MAR and BA) independently extracted the following relevant information related to the study characteristics: author(s) name; year

of publication; study period; study region; type of TB patients; study design; molecular DST method(s); sample size; total positive cases; total Mtb isolates for which DST was performed; frequency of any anti-TB drug resistance, any INH or RIF resistance, and MDR-TB; and gene mutations associated with RIF and INH resistance (Table 1). Lists of the gene mutations and specific nucleotide (codon) changes in each resistant gene probe assessed in this review are provided in Supplementary Tables S4 and S5.

2.7. Outcomes of interest

This systematic review and meta-analysis estimated the pooled prevalence of gene mutations conferring RIF and INH resistance in Mtb in Ethiopia. The frequency of any anti-TB drug resistance and resistance to INH and RIF were extracted from each included study. The frequency of each resistant gene mutations was counted out of the total resistant Mtb isolates for a particular anti-TB drug. Similarly, the rate of each nucleotide (codon) change at each resistant gene locus/probe (*rpoB, katG* and *inhA*) was calculated out of the total resistant genes. The pooled estimate of the prevalence of nucleotide (codon) changes at each resistant gene loci/probe was measured. We also estimated the pooled prevalence of gene mutations or the absence of bands at each wild-type (WT) probe of the *rpoB, katG* and *inhA* genes (Supplementary Table S5).

2.8. Data processing and statistical analysis

Relevant data were extracted from the included studies using a standard format prepared in Microsoft Excel and the data were exported into Stata Statistical Software: Release 11 (StataCorp LP, College Station, TX, USA) for further analysis. Using the binomial distribution formula, the standard error was calculated for each study. Considering the variation in true effect sizes across a population, a Der Simonian–Laird random-effects model was performed for the analyses at a 95% confidence level. The heterogeneity of studies was determined using Cochrane's Q statistics (χ^2), inverse variance (I^2) and P-values. Publication bias across the studies was measured through Egger's regression test [30] and was displayed using funnel plots of the standard error of the logit event rate. A P-value of <0.05 was considered statistically significant.

Results

3.1. Search results

As shown in Fig. 1, a total of 960 potential studies were documented from the searched electronic database sources. Of the total articles, 760 were non-duplicate and were subjected to further evaluation, of which 452 were excluded based on their title and abstract, while 308 papers were retained for full-text article review. After full-text evaluation, 19 studies on the prevalence of gene mutations associated with RIF- and INH-resistant Mtb in Ethiopia were include in the final analysis.

3.2. Characteristics of included studies

As described in Table 1 and Supplementary Table S5, a total of 19 studies with 5057 TB patients were included in the final analysis [31–49]. Regarding the regional distribution, five studies each were from Addis Ababa [31,39,41,42,49], Amhara region [32,36,37,43,46] and Oromia region [33,40,44,45,48], while two studies were from South Nation Nationality and People's region [35,47], one study was from Somalia region [38] and one study was performed in multiple regions [33]. Regarding study design, 14 studies were cross-sectional [31–39,42,43,45,46,49]. Eight studies [31,33,38–40,45,46,48] assessed the RIF and INH resistance rate among PTB patients, five studies [35–37,44,49] assessed EPTB patients, and three studies [32,34,47] included both EPTB and PTB patients. GenoType® MTBDR*plus* assay and Xpert® MTB/RIF assay were the most common molecular DST methods used [31–34,36–49]. The resistance rate of Mtb to any anti-TB drugs, MDR-TB and resistance to RIF and INH was calculated out of a total of 3406 Mtb isolates in which DST was performed [31–49]. In total, 17 studies evaluated the prevalence of any INH resistance [31–35,37–43,45–49], while any RIF resistance [31–34,36–47,49] was evaluated among 3406 TB patients. All included studies except one reported the prevalence of MDR-TB strains [31–34,36–49]. Moreover, 17 studies [31–34,36–47,49] quantified the frequency of *rpoB* gene mutations and nucleotide (codon) changes in the 81-bp β-subunit of the *rpoB* gene among 847 RIF-resistant Mtb isolates, while 16 studies [31–35,37–43,45–47,49] reported the frequency of *katG* gene mutations among 949 INH-resistant Mtb isolates. Besides, 10 studies [31–34,38,42,43,45,46,48] reported mutations in the *inhA* promoter region, while four studies [31,32,42,43] reported the co-occurrence of *inhA* and *katG* genes among INH-resistant Mtb isolates.

3.3. Prevalence of any rifampicin (RIF) or isoniazid (INH) resistance in Mycobacterium tuberculosis isolates

Overall, 5057 pooled TB suspected patients were tested to identify MDR-TB, RIF and INH resistance patterns [31–49]. The prevalence of any anti-TB drug resistance among all diagnosed TB patients was 31.3% (1066/3406), while the prevalence of any RIF and any INH resistance was 24.9% (847/3406) and 27.9% (949/3406), respectively. Moreover, the prevalence of MDR-TB was 22.2% (755/3406) (Supplementary Fig. S1; Supplementary Table S5). The prevalence of any anti-TB drug resistance varied across the studies and geographical locations of Ethiopia. From the included studies, seven studies reported a higher prevalence of any anti-TB drug resistance ranging from 18.0% to 73.6% [31,32,38,41–43,45]. The prevalence of any INH-resistant Mtb ranged from 0.8% to 72.2%, while the prevalence of any RIF-resistant Mtb ranged from 1.3% to 68.1% [31–34,36–47,49] (Table 1; Supplementary Table S5).

3.4. Frequency of rpoB, katG and inhA promoter mutations

A total of 949 Mtb strains with any INH resistance were identified by standard WHO-approved molecular diagnostic methods, among which a higher proportion of mutations was detected in the *katG* gene (95.8%; 909/949) compared with the *inhA* promoter region (5.9%; 56/949). In INH-resistant Mtb strains, the most common mutations were observed in the *katG*MUT1 probe (860 cases) and *katG*WT probe (309 cases). In the *inhA* promoter region, the most frequent mutations were observed in the *inhA*MUT1 probe (*inhA* C15T; 31 cases), followed by *inhA*WT1 probe (30 cases) and *inhA*WT2 probe (23 cases), and finally *inhA*MUT2 probe (10 cases). The frequency of mutations in *inhA*MUT3A and MUT3B was 8 cases, respectively. A total of 34 Mtb strains had mutations in both *katG* and the *inhA* promoter region (Table 1; Supplementary Table S5).

Moreover, a total of 847 Mtb strains with any RIF resistance were identified, among which the frequency of mutation in the *rpoB* gene was 90.8% (769/847). In RIF-resistant Mtb strains, the most common mutations were found in the *rpoB*MUT3(S531L) probe (550 cases), followed by the *rpoB*WT8 probe (224 cases) and *rpoB*WT7 probe (91 cases), while other gene mutations were observed in *rpoB*MUT2A(H526Y) (68 cases), *rpoB*MUT2B(H526D) (40 cases) and *rpoB*MUT1(D516V) (25 cases). Moreover, 10 RIF-resistant Mtb strains showed *rpoB* gene mutations at codons 447–452, while

Table 1
Characteristics of studies included in the systematic review and meta-analysis

Author(s)	Publication year	Study period	Study region	Type of patients	Study design	Molecular diagnostic method(s)	Patients (participants) (*n*)	Total positive cases (*n*)	Total isolates with DST performed (*n*)	Any drug resistance (*n*)	Any INH or RIF resistance (*n*)		MDR-TB (*n*)	Anti-TB drug resistance mechanisms (*n*)	Frequency of gene mutations (*n*)				
														rpoB	*katG*	*inhA*	*katG*+*inhA*	*rpoB*+*katG*	
											INH	RIF							
Abate et al. [31]	2014	2012–2013	AA	PTB	Retrospective cross-sectional	GenoType® MTBDR*plus*	736	736	736	523	481	470	427	*rpoB*: S531L (323), H526Y (31), H526D (19), D516V (13), *rpoB*WT2 (3), *rpoB*WT3 (8), *rpoB*WT7 (28), *rpoB*WT4 (21), *rpoB*WT6 (2), *rpoB*WT8 (12), *rpoB* mixed mutations at different codon positions (10); *katG*: S315T1 (447), S315T2 (5), *katG*WT mutation (315)-unknown codon (9), *katG* mixed mutations (S315T) (4); *inhA*: C15T (5), *inhA*WT2mutation (1), *katG*MUT1+*inhA*MUT1 (S315T+C15T) (9), *katG*MUT1+*inhA*MUT2 (S315T+A16G) (1)	470	469	6	10	0
Alelign et al. [32]	2019	2015–2017	AM	PTB and EPTB	Cross-sectional	GenoType® MTBDR*plus*	111	111	111	20	20	2	2	*rpoB: rpoB*WT6 (2), *rpoB*WT8 (2); *katG:* S315T1 (20), *katG*WT (6); *inhA*WT1 (20), *inhA*WT2 (20)	2	20	20	20	20
Bedewi Omer et al. [33]	2016	2012–2013	OR	PTB	Cross-sectional	GenoType® MTBDR*plus*	279	279	279	31	25	9	3	*rpoB:* S531L (3), H526Y (2), *rpoB*WT8 missed (4); *katG:* S315T1 (22), *katG*WT (3); *inhA*: C15T (2), *inhA*WT2 missed (1)	9	25	3	NR	NR
Bekele et al. [34]	2018	2006–2010	AA, AM, OR, SNNP	PTB and TBLN	Cross-sectional	GenoType® MTBDR*plus*	950	161	161	14	12	7	5	*rpoB*: D516V (8), S531L (3), H526Y (7), H526D (7), *rpoB*W8 missed (4); *katG*: S315T2 (8), S315T1 (3), *katG*WT missed (8); *inhA:* C15T (6), A16G (7), T8C (8), T8A (7), *inhA*WT1 (2)	8	8	8	NR	NR
Beyene et al. [35]	2009	2005–2006	SNNP	EPTB	Cross-sectional	MLPA	171	156	95	11	11	NR	NR	*katG:* S315T (11)	NR	11	NR	NR	NR
Biadglegne et al. [36]	2014	NR	AM	EPTB	Cross-sectional	Xpert® MTB/RIF and GenoType® MTBDR*plus*	231	32	32	3	NS	3	3	*rpoB:* S531L (2), #*rpoB*WT3 (deletion) (1)	3	NR	NS	NS	NR
Biadglegne et al. [37]	2013	2012	AM	EPTB	Cross-sectional	GenoType® MTBDR*plus* and GenoType® MTBDR*sl*	226	226	226	13	8	3	2	*rpoB*: S531L (2), Q513L (1), *rpoB*WT3 (1), *rpoB*WT8 (2); *katG*: S315T (8), *katG*WT (8)	3	8	0	NR	2
Brhane et al. [38]	2017	NR	SO	PTB	Cross-sectional	GenoType® MTBDR*plus*	105	98	98	18	18	10	10	*rpoB*: S531L (8), H526TY (1), H526D (1); *katG:* S315T1 (15), *katG*WT (15); *inhA:* C15T (3)	8	15	3	NR	NR

(*continued on next page*)

Table 1 (*continued*)

Author(s)	Publication year	Study period	Study region	Type of patients	Study design	Molecular diagnostic method(s)	Patients (participants) (*n*)	Total positive cases (*n*)	Total isolates with DST performed (*n*)	Any drug resistance (*n*)	Any INH or RIF resistance (*n*)		MDR-TB (*n*)	Anti-TB drug resistance mechanisms (*n*)	Frequency of gene mutations (*n*)				
														rpoB	*katG*	*inhA*	*katG*+*inhA*	*rpoB*+*katG*	
Damena et al. [39]	2019	2015–2016	AA	PTB	Cross-sectional	GenoType® MTBDR*plus* and GenoType® MTBDR*sl*	213	150	150	20	20	16	16	*rpoB:* S531L (13), D516V (1), H516Y (1), H516D (3), *rpoB*WT87 (13), *rpoB*WT7 (2); *katG:* S315T1 (20), *katG*WT (19)	15	20	0	0	15
Haile et al. [40]	2019	2015–2016	OR	PTB		GenoType® MTBDR*plus*	111	92	92	6	5	1	0	*rpoB*: S531L (1), *rpoB*WT8 (1); *katG*: S315T1 (5), *katG*WT (5)	1	5	0	0	0
Kebede et al. [41]	2017	2011–2012	AA	MDR-TB	NR	GenoType® MTBDR*plus*	72	72	72	53	52	49	48	*rpoB:* S531L (40), D516V (2), H526Y (4), *rpoB*WT8 (35), *rpoB*WT2 (1), *rpoB*WT4 (2), *rpoB*WT3 (4), *rpoB*WT7 (6); *katG:* S315T1 (52)	46	52	NR	NR	NR
Meaza et al. [42]	2017	2015	AA	MDR-TB	Cross-sectional	GenoType® MTBDR*plus* V.2.0	274	89	89	49	37	41	29	*rpoB*: S531L (27), H526Y (2), *rpoB*WT8 (27), *rpoB*WT7 (2), *rpoB*WT1 (1); *katG*: S315T/L (28); *inhA*: C15T (1), A16G (1), *inhA*WT1 (1), *inhA*WT2 (1)	27	28	1	1	NR
Mekonnen et al. [43]	2015	2012–2014	AM	MDR-TB	Cross-sectional	GenoType® MTBDR*plus*	413	413	413	195	176	169	150	*rpoB:* S531L (85), H526Y (19), H526D (7), *rpoB*WT2 (13), *rpoB*WT3 (33), *rpoB*WT4 (23), *rpoB*WT7 (39), *rpoB*WT8 (95); *katG:* S315T1 (155), Dele-315 (16), S315T2 (1), *katG*WT (172); *inhA:* C15T (8), *inhA*WT1 (8)	111	155	8	3	NR
Tadesse et al. [44]	2017	2013–2015	OR	EPTB	NR	GeneXpert® MTB/RIF	436	310	279	10	NS	10	10	*rpoB*: (codon 447–452) (10)	10	NS	NS	NS	NS
Tadesse et al. [45]	2016	2013–2014	OR	PTB	Cross-sectional	GenoType® MTBDR*plus* V.2	122	118	112	44	41	34	31	*rpoB:* S531L (28), H526D (1), *rpoB*WT7 (2), *rpoB*WT8 (31); *katG:* S315T1 (36), *katG*WT-315 (31); *inhA*: C15T (4), *inhA*WT (15/16) (4)	34	36	4	NR	NR
Tessema et al. [46]	2012	2009	AM	PTB	Cross-sectional	GenoType® MTBDR*plus* and GenoType® MTBDR*sl*	260	260	260	45	35	15	13	*rpoB*: S531L (11), H526D (1), *rpoB*WT8 (11), *rpoB*WT7 (1); *katG*: S315T (33), *katG*WT (33); *inhA*: C15T (2), *inhA*WT1 (2)	15	33	2	NR	NR
Wondale et al. [47]	2018	2014–2016	SNNP	PTB and EPTB	NR	GenoType® MTBDR*plus* V.2.0	161	126	126	4	1	3	1	*rpoB*: H526D (1), D516V (1); *katG*: S315T2 (1)	3	1	NR	NR	2
Workalemahu et al. [48]	2013	2011	OR	PTB	NR	GenoType® MTBDR*plus*	121	15	15	1	1	NR	0	*inhA* (1)[#]	NR	NR	1	NR	NR
Zewdie et al. [49]	2017	2014	AA	EPTB	Cross-sectional	GenoType® MTBDR*plus*	65	60	60	6	6	5	5	*rpoB:* S531L (4), H526Y (1), *rpoB*WT8 (4), *rpoB*WT7 (1); *katG:* S315T2 (1), S315T1 (5), *katG*WT (6)	5	6	0	0	5

AA, Addis Ababa; AM, Amhara; EPTB, extrapulmonary tuberculosis; MDR-TB, multidrug-resistant tuberculosis; MUT, mutant; NR, not reported; NS, not studied; OR, Oromia; PTB, pulmonary tuberculosis; SNNP, South Nations, Nationalities, and People's region; SO, Somalia; TBLN, tuberculosis lymphadenitis; WT, wild-type.
[#]Amino acid change not reported.

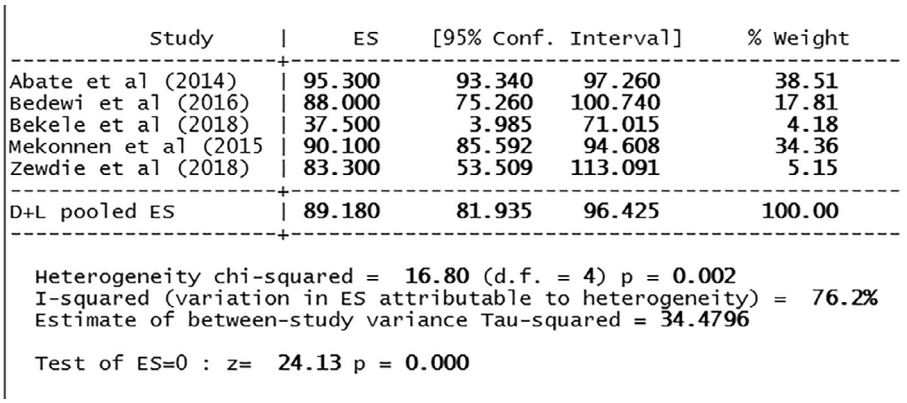

Fig. 2. Pooled prevalence of *katG*MUT1(S315T1) resistance among isoniazid-resistant *Mycobacterium tuberculosis* isolates.

Table 2
Results of mutation pattern of 949 isoniazid-resistant *Mycobacterium tuberculosis* isolates

Mutation(s)	Frequency			I^2 (*P*-value)
katG	*inhA*	No. of patients	% (95% CI)	
*katG*WT(315) absent	–	567	48.69 (–5.20 to 102.58)	99.5% (<0.001)
*katG*MUT1(S315T1)	–	700	89.18 (81.94–96.43)	76.2% (0.002)
*katG*MUT1(S315T2)	–	663	0.91 (0.195–1.63)	0.0% (0.466)
-	*inhA*WT1(15/16) absent	–	–	–
-	*inhA*WT2(8) absent	506	20.65 (–5.36 to 46.66)	0.0% (0.594)
-	*inhA*MUT1(C15T)	518	77.48 (57.84–97.13)	0.0% (0.848)
-	*inhA*MUT2(A16G)	–	–	–
-	*inhA*MUT3A(T8C)	–	–	–
-	*inhA*MUT3B(T8A)	–	–	–

CI, confidence interval.

the other strain had *rpoB* gene mutation at CAA/G→UUA/G(Q513L) (Table 1; Supplementary Table S5).

3.5. Meta-analysis

Of the 19 studies, 17 studies evaluated 949 genotypically resistant Mtb isolates for mutations in the *katG* gene inclusive of codon 315. This meta-analysis resulted a pooled prevalence of *katG*MUT1(S315T1) of 89.2% (95% CI 81.94–96.43%) with an I^2 of 76.2% and *P*-value of 0.002 (Fig. 2; Table 2). During our evaluation of publication bias, the funnel plot showed a symmetrical distribution. For Egger's regression test, the *P*-value was 0.071, indicating the absence of publication bias (Fig. 3). However, this review found a low pooled prevalence of *katG*MUT2(S315T2): 0.9% (95% CI 0.195–1.63%) with an I^2 of 0.0% and *P*-value of 0.466 (Table 2). Besides, the estimated pooled prevalence of the absence of band at the wild-type [*katG*WT(315)] was 48.7% (95% CI –5.20% to 102.58%) with an I^2 of 99.5% and *P*-value of <0.001(Table 2).

Mutations in the *inhA* promoter region were estimated and the meta-analysis resulted in a pooled prevalence of *inhA*MUT1(C15T) of 77.5% (95% CI 57.84–97.13%) with an I^2 of 0.0% and *P*-value of 0.848 (Fig. 4; Table 2). Similarly, the estimated pooled prevalence of the absence of band at wild-type *inhA*WT2(8) was 20.7% (95% CI –5.36% to 46.66%) with an I^2 of 0.0% and *P*-value of 0.594 (Table 2).

During the meta-analysis, 17 publications evaluated 847 genotypically RIF-resistant Mtb isolates for mutations in the *rpoB* gene inclusive of the 81-bp β-subunit ranging from codons 507–533, particularly at codons 526, 516 and 531. The highest estimated pooled prevalence of gene mutation associated with the *rpoB* gene was observed in *rpoB*MUT3(S531L) at 74.2% (95% CI 66.39–82.00%) with an I^2 of 64.6% and a *P*-value of 0.002 (Fig. 5; Table 3). Publication bias was evaluated using Egger's regression test revealed the *P*-value was 0.968, and a funnel plot showed a symmetrical distribution, indicating the absence of publication bias

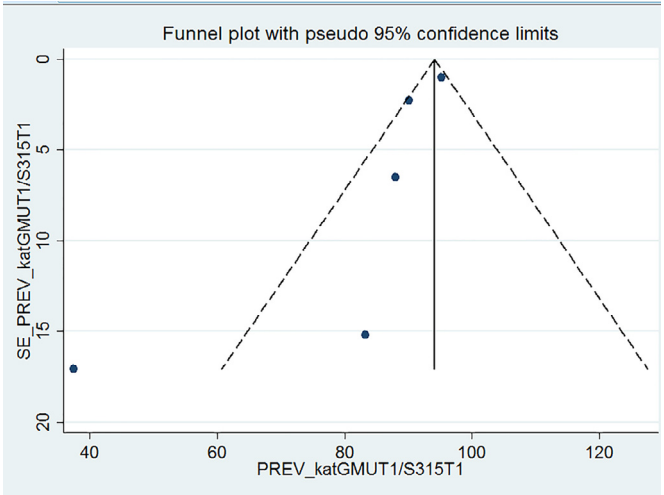

Fig. 3. Funnel plot for publication bias. Prevalence (PREV) represented on the *x*-axis and standard error (SE) of the prevalence of *katG*MUT1(S315T) on the *y*-axis.

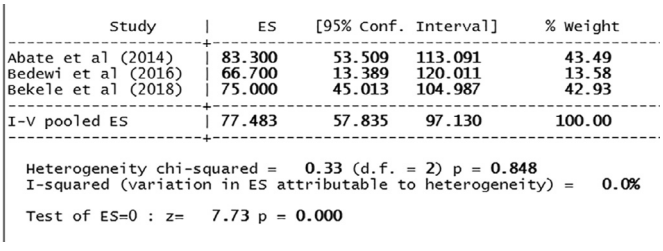

Fig. 4. Pooled prevalence of *inhA*MUT1(C15T) resistance among isoniazid-resistant *Mycobacterium tuberculosis* isolates.

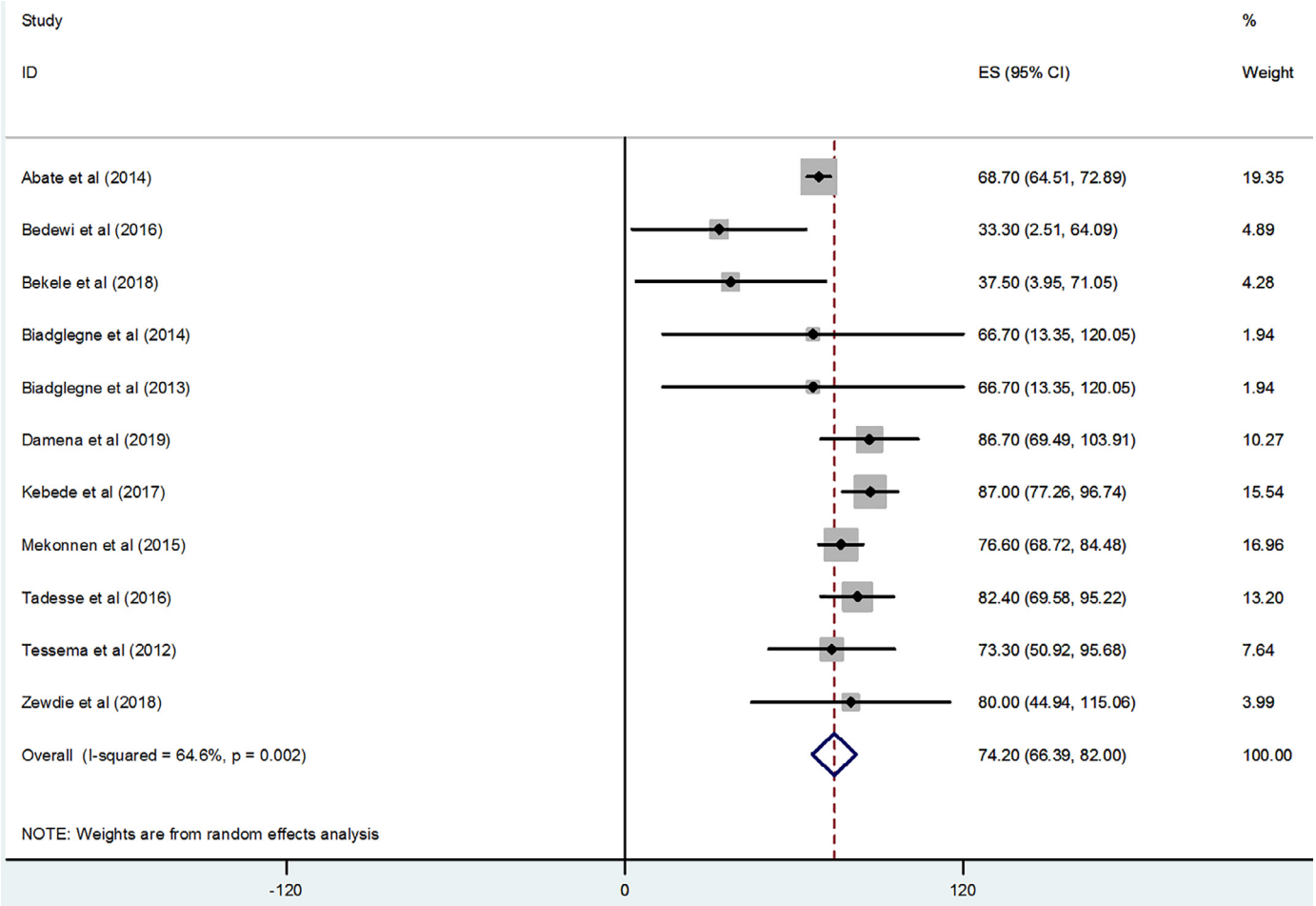

Fig. 5. Forest plot of the pooled prevalence of *rpoB*MUT3(S531L) resistance among rifampicin-resistant *Mycobacterium tuberculosis* isolates.

Table 3
Results of mutation patterns of 847 rifampicin-resistant *Mycobacterium tuberculosis* isolates

rpoB mutation	Frequency		I^2 (*P*-value)
	No. of patients	% (95% CI)	
*rpoB*WT1 absent	–	–	–
*rpoB*WT2	688	4.12 (−1.24 to 9.48)	85.2% (0.001)
*rpoB*WT3	645	19.92 (−3.37 to 43.21)	93.1% (<0.001)
*rpoB*WT4	688	9.20 (0.87–17.54)	88.1% (<0.001)
*rpoB*WT5	–	–	–
*rpoB*WT6	485	0.41 (−0.17 to 1.00)	0.0% (0.328)
*rpoB*WT7	799	19.30 (8.78–29.82)	90.5% (<0.001)
*rpoB*WT8	806	58.21 (26.38–90.04)	99.1% (<0.001)
*rpoB*MUT1(D516V)	538	2.96 (1.53–4.39)	0.0% (0.503)
*rpoB*MUT2A(H526Y)	776	17.20 (8.25–26.15)	85.7% (<0.001)
*rpoB*MUT2B(H526D)	724	13.91 (5.80–22.02)	87.5% (<0.001)
*rpoB*MUT3(S531L)	780	74.20 (66.39–82.00)	64.6% (0.002)

CI, confidence interval.

(Fig. 6). Moreover, the pooled prevalence of *rpoB*MUT2A(H526Y) was 17.2% (95% CI 8.25–26.15%) with an I^2 of 85.7% and a *P*-value of <0.001 (Fig. 7; Table 3). The funnel plot showed symmetrical distribution, and in Egger's test the *P*-value was 0.107, which indicated there was no publication bias (Fig. 8). The analysis derived a pooled *rpoB*MUT2B(H526D) and *rpoB*MUT1(D516V) prevalence of 13.9% and 2.96%, respectively (Table 3).

Discussion

Global TB control and prevention is challenging due to the emergence of drug-resistant bacilli [1,2,50], particularly due to the evolutionary path of MDR-TB [1,8]. Use of molecular-based diagnostic methods, with the detection of mutations in specific genes associated with anti-TB drug resistance, is more efficient and effective. Utilisation of these methods in clinical microbiology laboratories could reduce the turnaround time required to diagnose cases from weeks to hours [51]. Detection of gene mutations in resistance-determining regions in resistant Mtb isolates plays a crucial role in the rapid detection of anti-TB drug resistance and could aid strategies to further explore the mechanisms of resistance.

In this review, we assessed the prevalence of mutations in genes associated with RIF- and INH-resistant Mtb in Ethiopia. Our review demonstrated a prevalence of 95.8% for *katG*315 mutation and 5.9% for *inhA* promoter region mutation in patients with

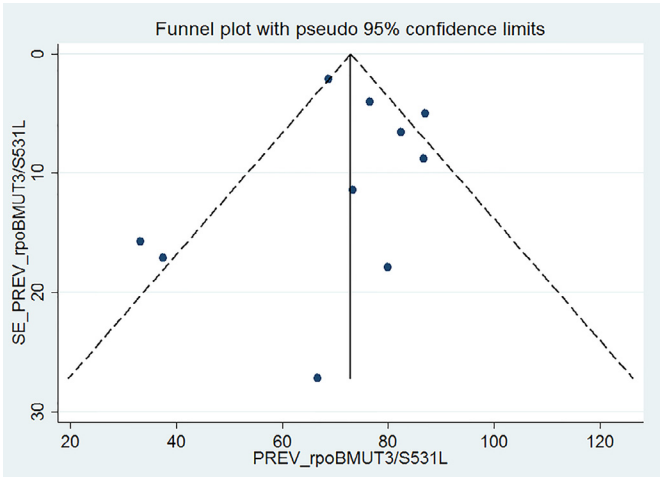

Fig. 6. Funnel plot for publication bias. Prevalence (PREV) represented in the *x*-axis and standard error (SE) of the prevalence of *rpoB*MUT3(S531L) on the *y*-axis.

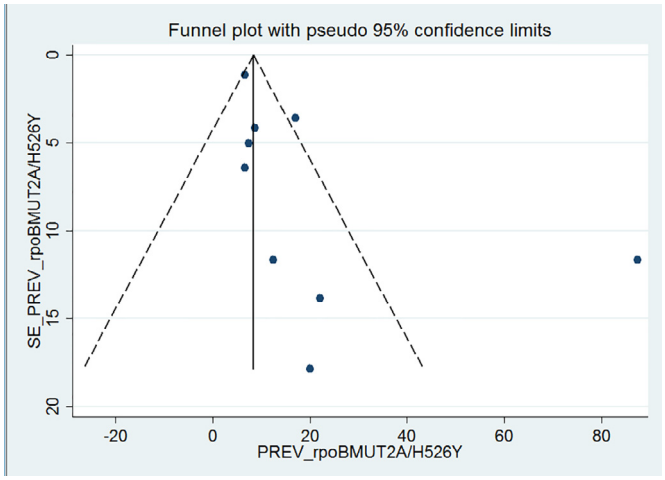

Fig. 8. Funnel plot for publication bias. Prevalence (PREV) represented in the *x*-axis and standard error (SE) of the prevalence of *rpoB*MUT2A(H526Y) on the *y*-axis.

INH-resistant Mtb, suggesting a major epidemic of DR-TB among these patients in Ethiopia. Moreover, the meta-analysis estimated a pooled prevalence of *katG*MUT1(S315T1) of 89.2%, while the estimated pooled prevalence of *inhA*MUT1(C15T) in the *inhA* promoter region was 77.5%. In agreement with our findings, an earlier systematic review found that S315T mutations (79.1%) in the *katG* gene and C15T mutations (4.5%) in the *inhA* promoter region were responsible for INH resistance in Mtb isolates from Ethiopia [52]. Supporting this finding, a study conducted in India (the first high-TB and MDR-TB burden country in the world) reported that, of the total tested isolates, 71.0% had a detectable mutation in *katG*315 and 29.0% in the *inhA* promoter region. A similar report revealed that the estimated pooled prevalence of *katG*MUT1(S315T1) was 63.2% [12]. Few studies conducted in Africa have produced sup-

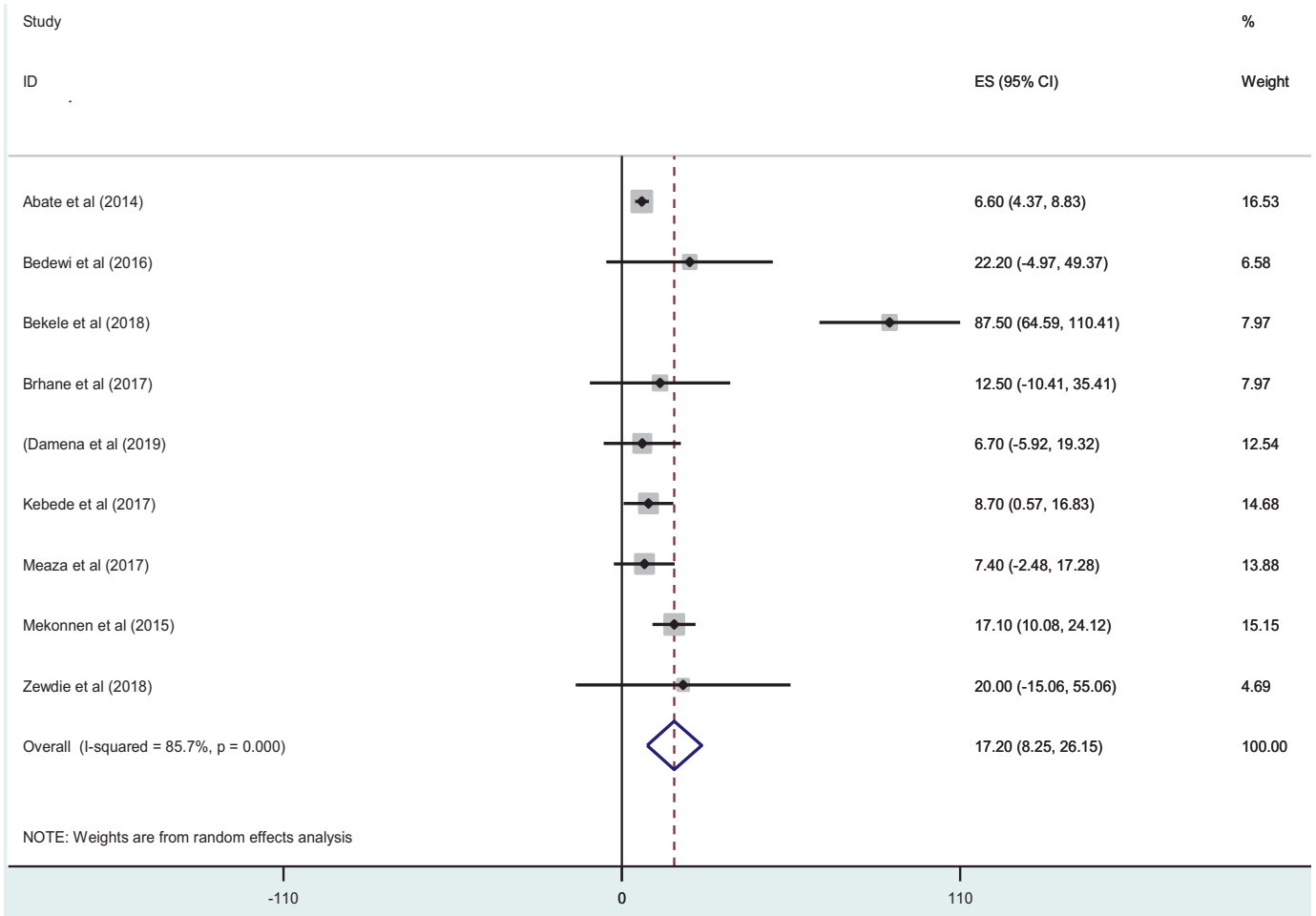

Fig. 7. Forest plot of the pooled prevalence of *rpoB*MUT2A(H526Y) resistance among rifampicin-resistant *Mycobacterium tuberculosis* isolates.

porting evidence. A study conducted in Uganda revealed that the *katG* and *inhA* gene mutations were mainly due to S315T (76%) and C15T (8%) nucleotide changes, respectively [11]. A recent study conducted in Monrovia, Liberia, revealed that the estimated global frequencies of *katG*315 and *inhA*–15 were suggestively higher at 86% and 34%, respectively [53]. Approximately 64% of phenotypic resistance to INH globally is attributed to the *katG*(S315T) mutation [8]. Similarly, a study conducted in the USA estimated the global frequency of the *katG*315 gene mutation to be 85%, and 17% for *inhA*–15, while the cumulative frequency was 91% [54]. This analysis revealed a robust association between the percentage of INH resistance-conferring mutations due to *katG*(S315T) evaluated in clinical isolates and many different indicators of TB transmission intensity, supporting the suggestion that gene mutation at the 315 codon position of *katG* confers high-level INH resistance in Mtb without reducing virulence or transmissibility.

In the case of RIF-resistant isolates, our study demonstrated that the most common gene mutation associated with RIF resistance was observed at *rpoB*MUT3(S531L) with a pooled prevalence of 74.2%. In agreement with this finding, a study conducted in Sudan revealed that the prevalence of gene mutation at codon 531 was 64.1% [14]. The same report noted that all genetic alterations were single base substitutions and the most common mutation was observed at codon S531L [14]. This mutation was previously reported at a prevalence of 70% by an earlier study in Uganda [55]. Similarly, a study conducted in Morocco showed that the most common mutation in the *rpoB* gene was substitution at codon 531 (S531L), accounting for 46.2% [56]. In line with our findings, worldwide RIF-resistant Mtb isolates are spreading widely with S531L mutation. Several other previous studies conducted in developed countries revealed a predominance of S531L in RIF-resistant Mtb isolates, e.g. 67.2% in Mexico [57] and Georgia [58], 41.2% in Latvia [59], 59.5% in Italy [60], 56% in Greece [61] and 43% in Japan [62].

The second most frequent RIF resistance-conferring mutation in this review was observed at *rpoB*MUT2A(H526Y) with an estimated pooled prevalence of 17.2%. Similarly, a study conducted by Elbir et al. in Sudan reported that the frequency of gene mutation at codons 531 and 526 were 64.1% and 17.9%, respectively [14]. Several previous studies have reported that the most common gene mutation associated with RIF resistance in Mtb was due to nucleotide (codon) change at 531 and 526 in the *rpoB* gene [11,14,50,63]. More than 95% of RIF-resistant isolates possess mutations within a hypervariable region of the *rpoB* gene encoding the β-subunit of RNA polymerase [64,65], and the most common mutations observed are S531L, H526D and D516V [11,66].

An understanding at the molecular level of the mechanism of drug resistance in Mtb will enable us to develop improved diagnostic tools. It deserves further investigation to determine which gene mutations may play a critical role in the epidemic of drug-resistant Mtb, particularly MDR-TB, to inform local TB control as well as to determine MDR-TB strategies in the country [9]. It is becoming increasingly clear that the performance of rapid molecular tests in TB cannot be extrapolated from one setting to another but needs to be validated in each geographic setting [67] through systematic surveys to ascertain the mutation profiles and frequencies in geographic regions where the tests are being deployed [8,54].

This review had some limitations. Only published articles in the English language were included in the analysis. Due to lack of detailed information in the few included studies, the review did not assess the prevalence of RIF and INH monoresistance nor estimate the pooled prevalence of gene mutations associated with RIF and INH monoresistance. The majority of studies have not explained the proportions of gene mutations based on sex and age of participants, so this review was unable to assess sex- and age-wise comparison of mutations associated with RIF and INH resistance. The development of gene mutations in drug-resistant Mtb strains varies across different treatment outcomes (failure, loss of follow-up, re-treatment cases); however, due to lack of detailed information regarding these issues, this review failed to determine the estimated pooled prevalence of different gene mutations conferring RIF and INH resistance among those TB patient cohorts.

In conclusion, RIF resistance most commonly occurred due to mutations in *rpoB*MUT3(S531L), followed by *rpoB*MUT2A(H526Y), while INH resistance most frequently occurred due to mutations in the *katG*315 gene, and these mutations were also associated with multidrug resistance and polydrug resistance. Commonly, mutations both in the *katG* gene and *inhA* promoter region increase the development of MDR-TB and the risk of relapse. However, increasing frequencies of these gene mutations appears to vary by region, which could lead to differences in the sensitivity of molecular diagnostics tools if the tests are based only on these gene mutations. This would permit modifying molecular tests to specific geographical locations, better interpretation of the molecular tests being used, and better therapy recommendations.

Availability of data and materials

The data sets analysed during this review are available from the corresponding author upon reasonable request.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version.

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Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.06.009.

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