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Spoligotyping and drug sensitivity of *Mycobacterium tuberculosis* isolated from pulmonary tuberculosis patients in the Arsi Zone of southeastern Ethiopia

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Abstract

Tuberculosis (TB) is one of the leading causes of morbidity and mortality in different zones of Ethiopia. This study was undertaken to identify the strains of *Mycobacterium tuberculosis* and evaluate their drug sensitivity profiles in the Arsi Zone. A total of 111 isolates of *M. tuberculosis* from individuals with pulmonary TB were included and speciation and strain identification were performed using Region of difference 9 and spoligotyping, respectively. The drug sensitivity patterns were assessed using Bactec MGIT 960 SIRE and GenoType MTBDRplus line probe assays. Of 111 isolates, 83% were interpretable and 56 different spoligotype patterns were identified. From these, 22 patterns were shared types while the remaining 34 were orphans. The predominant shared types were spoligotype international type (SIT) 149 and SIT53, comprising 12 and 11 isolates, respectively. Euro-American lineage was the dominant lineage followed by East-African-Indian. Phenotypically, 17.2% of tested isolates were resistant to any first-line drugs and 3.1% were multidrug-resistant. Higher (6.2%) mono-resistance was observed to streptomycin, and no resistance was observed to rifampicin or ethambutol. Genotypically, five (5.4%) isolates were resistant to isoniazid and mutated at codon S315T1 of *katG*. In contrast, only 1.1% of the isolates were resistant to rifampicin and were mutated at codon S531L of *rpoB* gene. In this study, a high proportion of orphan strains were isolated, which could suggest the presence of new strains and a high percentage of mono-resistance, warranting the need to strengthen control efforts.

Keywords: Drug sensitivity, Ethiopia, Mutation, *Mycobacterium tuberculosis*, Spoligotyping
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Introduction

Tuberculosis (TB) is the leading cause of death from a single infectious agent, ranking above human immunodeficiency virus (HIV)/AIDS [1]. Globally, there were an estimated 10.0 million incident cases of TB and 87% of these occurred in 30 high-TB-burden countries [1]. TB affects all countries and all age groups, but the epidemiological distribution of TB cases is heavily skewed towards low-income countries [1]. Pandemics of HIV/AIDS, deterioration of public health systems and emergence of multidrug-resistant (MDR) forms of TB have worsened the spread of TB in developing countries [2].

According to the WHO report [1], Ethiopia is one of the world's 30 high-TB-burden countries with an estimated incidence of 164 new TB cases per 100 000 population. The country contains 2.7% newly diagnosed and 14% previously treated individuals with multidrug-resistant (MDR) TB [1]. As in other developing countries, TB/HIV co-infection and the emergence of MDR-TB strains are becoming pressing challenges in the efforts

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to control TB in Ethiopia [1,3,4]. All recent data shows that TB and drug-resistant forms of TB remain a major public health concern in Ethiopia.

Recent advances in molecular typing techniques highlighted the substantial genetic diversity of *Mycobacterium tuberculosis* complex isolated from individuals with TB [5]. Genetic variability can be translated into phenotypic differences in transmission capacity, virulence and drug susceptibility, which may further give rise to diversity in disease outcome and epidemiological variation among different geographic regions [6,7]. Earlier genotyping studies in Ethiopia have also indicated considerable genetic variation among clinical isolates of *M. tuberculosis* [8,9].

Knowledge on the strain diversity of *M. tuberculosis* helps to elucidate the patterns and dynamics of TB transmission [10]. In addition to the possible impact of strain variation on the outcome of TB infection and disease, the diversity of strains is relevant for our understanding of drug resistance [11].

On the other hand, drug-resistant TB remains a significant challenge in TB treatment and control programmes worldwide [12]. A report from Ethiopia revealed that the control effort of TB was threatened by the rapid emergence of drug-resistant strains, particularly MDR-TB [3,4]. A population-based study conducted in the Hitossa District of Arsi zone showed high rates of prevalence for primary and secondary resistance to any of the first-line anti-TB drugs and MDR-TB [13].

Although a few genotypic studies have been undertaken for strain identification from different part of Ethiopia, little is known about the strains and lineages of *M. tuberculosis* circulating in the Arsi Zone. Moreover, the drug sensitivity profiles of these strains were not known. The objective of this study was to identify the strains and lineages of *M. tuberculosis* isolated from the Arsi Zone, and also to evaluate their drug sensitivity profiles to the first-line anti-TB drugs using phenotypic and genotypic methods.

Materials and methods

Source of the isolates and processing

A total of 111 *M. tuberculosis* isolates were obtained from the TB laboratory of the Aklilu Lemma Institute of Pathobiology. These culture-positive isolates were originally isolated from individuals with pulmonary TB from the Arsi Zone between April 2015 and March 2016 and were stored at −80°C at the Institute for further molecular investigation and drug susceptibility tests. All the laboratory tests were performed at the TB laboratories of the Aklilu Lemma Institute of Pathobiology and the Ethiopian Public Health Institutes. These laboratories provide research services, TB diagnoses and drug susceptibility tests. The sociodemographic data of the individuals from whom the isolates were recovered were collected using a case-reporting form. The study protocol was approved and ethically cleared by Addis Ababa University, Aklilu Lemma Institute of Pathobiology Institutional Review Board (ref. no. ALIPB/IRB/001/2017/18).

Sub-culturing of the isolates

The frozen isolates were retrieved from −80°C and thawed at room temperature. The colony suspension was homogenized by gentle mixing and sub-cultured on solid Lowenstein–Jensen medium. The specimens were incubated at 37°C and checked weekly for up to 8 weeks. For the purposes of obtaining fresh specimens for phenotypic drug susceptibility tests, we also sub cultured the isolates on BACTEC™ MGIT™ liquid media following the manufacturer's instructions and FIND manual (Becton Dickinson, Sparks, MD, USA).

Briefly, 0.8 mL of PANTA antibiotic mixture and OADC enrichment (Becton Dickinson) was added to MGIT 960 tubes before inoculation, then 0.5 mL of the processed specimen was inoculated into each tube. After inoculation, all the inoculated MGIT tubes were placed in the MGIT 960 instrument and incubated until the instrument flagged them as positive or negative by its indicator lights. MGIT tubes that gave a positive fluorescent signal with the BACTEC MGIT 960 equipment were checked for acid-fast bacilli using Ziehl–Neelsen staining, whereas tubes that failed to show any growth after 42 days of incubation in the machine were removed and classified as negatives.

DNA extraction

DNA was released from the bacterium by suspending two loopfulls of mycobacterial colonies into 200 µL of sterile distilled water, and thereafter by heating the bacterial suspension in the water bath at 80°C for 60 min. Then, the solution was centrifuged at 3000 rpm for 2 min, after which the supernatant was collected and stored at −20°C for molecular typing and/or other related investigations.

Region of difference 9

Region of difference 9 (RD9) deletion typing was performed on the DNA of heat-killed isolates to confirm the presence or absence of RD9 as previously described by Brosch et al. [14]. The three primers used were RD9flankF, RD9IntR and RD9flankR, each at a concentration of 100 µM. The PCR amplification was performed in a total of 20 µL, reaction mixture consisting of 10 µL HotStarTaq Master Mix (Qiagen, Crawley, UK), 7.1 µL distilled water, 0.3 µL each of the three primers (100 mM) and 2 µL DNA template (heat killed). The reaction was heated for 10 min at 95°C for enzyme activation

followed by 35 cycles of 1 min of denaturation at 95°C, 0.5 min of annealing at 61°C and 2 min of extension at 72°C, and then a final extension at 72°C for 10 min. Thereafter the product was removed from the thermocycler and run on agarose gel electrophoresis. For gel electrophoresis, 8 µL PCR products was mixed with 2 µL loading dye, loaded onto 1.5% agarose gel and electrophoresed at 100 V and 500 mA for 45 min. The gel was then visualized using a computerized Multi-Image Light Cabinet (VWR). *Mycobacterium tuberculosis* H37Rv and *Mycobacterium bovis* Bacillus Calmette–Guérin were used as positive controls, and water was use as a negative control. Detection of a band size of 396 bp was considered as positive for *M. tuberculosis* whereas a band size of 575 bp was considered to correspond to either *M. bovis* or *Mycobacterium africanum*.

Spoligotyping

Spoligotyping was performed following the method previously described by Kamerbeek et al. [15] and as per the spoligotype kit supplier's instructions (Ocimum Biosolutions, Ijsselstein, the Netherlands). The direct repeat (DR) region was amplified by PCR using oligonucleotide primers DRa (GGTTTTGGGTCTGACGAC) and DRb (CCGAGAGGGGACGGAAAC), which were derived from the DR sequence. DNA from known strains of *M. bovis* SB 1176 and *M. tuberculosis* H37Rv were used as positive controls, whereas Qiagen water (Qiagen, Hilden, Germany) was used as a negative control. A total volume of 25 µL of reaction mixture was prepared, consisting of 12.5 µL of Hot StarTaq Master Mix, 2 µL of each of the two primers (20 pmol each), 5 -µL suspension of heat-killed cells (approximately 10–50 ng), and 3.5 µL distilled water. The mixture was heated for 15 min at 96°C and then subjected to 30 cycles of 1 min at 96°C, 1 min at 55°C and 30 s at 72°C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR locus. After hybridization, the membrane was washed twice for 10 min in 2× SSPE and 0.5% SDS at 60°C and then incubated in 1:4000 diluted streptavidin-peroxidase (HotStar, Crawley, UK) for 45–60 min at 42°C. The membrane was washed twice for 10 min in 2× SSPE and 0.5% SDS at 42°C and rinsed with 2× SSPE for 5 min at room temperature. After the hybridizing, DNA was detected by the enhanced chemiluminescence method (Amersham Biosciences, Little Chalfont, UK) and by exposure to X-ray-film (Hyper film ECL, Amersham Biosciences) as specified by the manufacturer. The hybridization patterns were converted into binary and octal formats and compared with previously reported strains in the recent SITVIT2 database.

Drug susceptibility test

The phenotypic drug sensitivity test was performed using the BACTEC MGIT 960 system (Becton Dickinson Diagnostic Systems) SIRE kit as recommended by the manufacturer's instructions. The final critical drug concentrations of 0.1 µg/mL for isoniazid,1.0 µg/mL for rifampicin, 1.0 µg/mL for streptomycin and 5.0 µg/mL for ethambutol were used as described by Siddiqi and Rüsch-Gerdes [16]. Growth was monitored by the BACTEC 960 instrument, which automatically interprets results as susceptible or resistant. The *M. tuberculosis* H37Rv was run per batch of drug susceptibility tests set for quality control purposes. In this study, any drug resistance was defined as resistance to one and/or more first-line drugs, whereas mono-resistance was defined as resistance to only one of the four first-line drugs (isoniazid, rifampicin, streptomycin and ethambutol). MDR-TB was defined as *M. tuberculosis* strains that were resistant to at least isoniazid and rifampicin.

GenoType® MTBDRplus assays

Assays for the identification of mutations in the *rpoB* gene for rifampicin resistance, the *katG* gene for high-level isoniazid resistance, and the *inhA* gene for low-level isoniazid resistance were performed on the heat-killed mycobacterial culture according to the manufacturer's instructions (Hain Lifesciences, Nehren, Germany). The DNA of the standard strain H37Rv and molecular-grade water were used as positive and negative controls, respectively.

Data analysis

Sociodemographic data were analysed using the Statistical Package for Social Sciences (SPSS) version 20.0 (SPSS, Chicago, IL, USA). The descriptive statistics were used to depict the demographic variables and drug-resistant profiles of the patients, which were calculated as percentages of the study populations. All the generated spoligo patterns were entered and compared with the existing international, web-based SpolDB4 (SITVIT) database http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/to assign the shared type (SIT) and the web-based algorithm SPOTCLUST http://tbinsight.cs.rpi.edu/runspotclust.html was used to assign the lineages of each isolate.

Results

Demographic characteristics of patients

Sociodemographic data were collected from 111 individuals with smear-positive pulmonary TB. Of these 111 people, 64 (57.7%) were male and 47 (42.3%) were female. Their mean age was 32.53 years. One hundred and five (94.6%) of them had no previous history of TB treatment and six had a previous history of TB treatment (Table 1).

TABLE 1. Demographic characteristics of the patients

Variable		Number (%) of patients
Gender	Male	64 (57.7)
	Female	47 (42.3)
Age (years)	18–28	58 (52.3)
	29–39	25 (22.5)
	40–49	12 (10.8)
	>50	16 (14.4)
HIV status	Negative	61 (55.0)
	Positive	4 (3.6)
	Unknown	46 (41.4)
Marital status	Single	46 (41.4)
	Married	64 (57.7)
	Divorced	1 (0.9)
Previous history of treatment	New	105 (94.6)
	Previously treated	6 (5.4)

Speciation of *M. tuberculosis* complex

Out of 111 isolates, 92 (86.5%) had intact RD9 (396 bp), so were considered as *M. tuberculosis*. The remaining 19 (14.5%) isolates did not show an interpretable signal for RD9-based PCR.

Identification of strains

The spoligotyping of 92 mycobacterial isolates produced a total of 56 different spoligotype patterns with an overall diversity of 61%. Of these 56 patterns, 22 (39.3%) have been registered with the International Spoligotyping Database (SpolDB4) and hence were considered as 'shared types', whereas the remaining 34 (60.7%) patterns were not registered in the database and were therefore orphans. More than half (51%) of the isolates were clustered into 11 different spoligotype patterns while the remaining 49% were unique or singletons. The spoligotyping results are summarized in Tables 2 and 3.

The most commonly occurring clustered spoligotypes were SIT149 and SIT53, consisting of 12 (13.0%) and 11 (11.9%) isolates, respectively. The third and fourth most commonly observed shared types strains were SIT289 and SIT37, each comprising five (5.4%) isolates. On the basis of TB-insight RUN TB-lineages, four major lineages were identified: Euro-American, *M. africanum*, Indo-Oceanic and East-African-Indian. Euro-American was the dominant (79.3%) lineage followed by East-African-Indian (9.9%).

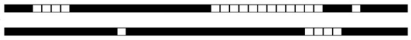
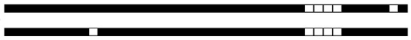
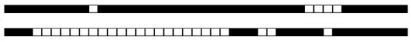
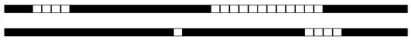
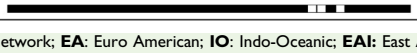
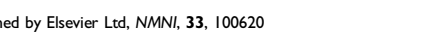
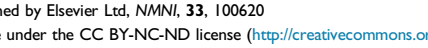
Phenotypic drug resistance

Bacterial growth was checked in MGIT 960 broth for a total of 92 isolates; 64 (70%) isolates showed growth, 22 (23.91%) did not. The cultures of six (6.52%) isolates were contaminated, so the drug susceptibility test was conducted on 64 isolates. Of the 64 isolates tested by MGIT for drug sensitivity, 11 were resistant to at least one first-line anti-TB drug and two isolates were MDR isolates. Resistance was highest to streptomycin (14.1%), followed by isoniazid (10.9%) (Table 4).

Genotypic detection for rifampicin and isoniazid resistance

The MTBDRplus assay was performed on 92 Lowenstein–Jensen culture-positive isolates. Of 92 *M tuberculosis* isolates tested, 86 (93.5%) were susceptible to the two most effective anti-TB drugs, and six (6.5%) were resistant to either rifampicin or isoniazid. Of the six resistant isolates, five

TABLE 2. Representative description of 57 shared international types (SIT) and corresponding lineages of *Mycobacterium tuberculosis* isolates from southeastern Ethiopia

SIT	No.isolates	Binary format	Octal code	CBN Lineage	KBBN sub-lineage	SPOTCLUST
149	12		777000377760771	EA	T3-ETH	T3
53	11		777777777760771	EA	T	T1
289	5		703777740003571	EAI	CAS1-Delhi	CAS
37	5		777737777760771	EA	T3	T1
118	2		777767777760771	EA	T	T1
121	2		777777775720771	EA	H3	Haarlem3
278	2		777777777760761	EA	T	T1
1166	2		777377777760771	EA	T	T1
343	2		700000007175771	M-africanum	AFRI	Family36
1547	2		777727777760771	EA	T3	T1
185	1		777766777760601	EA	X2	X2
26	1		703777740003771	EAI	CAS1-Delhi	CAS
40	1		777777377760771	EA	T	T1
52	1		777777777760731	EA	T2	T1
249	1		777640007760771	EA	T5-RUS1	T4
291	1		777777677760771	EA	T	T1
381	1		703777740003071	EAI	CAS1-Delhi	CAS
393	1		777757777760771	EA	T	T1
910	1		700000007177771	M-africanum	AFRI	Family36
1475	1		777357777760771	EA	T	T1
1675	1		703367400001771	EAI	CAS1-Kili	CAS
1690	1		777777777762771	EA	Manu2	Family33

CBN: a conformal Bayesian network; **EA**: Euro American; **IO**: Indo-Oceanic; **EAI:** East African-Indian.

TABLE 3. Representative description of 35 orphan *Mycobacterium tuberculosis* strains from southeastern Ethiopia

SIT	No.isolates	Binary format	Octal code	CBN Lineage	KBBN sub-lineage	SPOTCLUST
Orphan	1		400000037147671	M-africanum	AFRI	M. africanum
Orphan	1		701347740003661	EAI	CAS1-Delhi	CAS
Orphan	1		703377434001771	IO	CAS1-Kili	EAI4
Orphan	1		703377777243471	IO	Manu2	Family33
Orphan	1		703777777003771	IO	EAI	EAI5
Orphan	1		703777777743671	EA	Manu2	Family33
Orphan	1		715207637700061	EA	AFRI	Haarlem3
Orphan	1		715247437743771	EA	Manu2	Family33
Orphan	1		717377437741771	EA	Manu2	T1
Orphan	1		737377677760771	EA	T	T1
Orphan	1		757347637740661	EA	T	T1
Orphan	1		757347777767761	EA	Manu2	Family33
Orphan	1		757367737740621	EA	T	T1
Orphan	1		757377437743771	IO	Manu2	Family33
Orphan	1		775347777420731	EA	H3-Ural-1	Haarlem3
Orphan	1		776337407760771	EA	LAM5	LAM9
Orphan	1		777000007760771	EA	T3-ETH	T4
Orphan	1		777000037357661	M-africanum	PINI1	Family33
Orphan	1		777000177760771	EA	T3-ETH	T3
Orphan	1		777007377760771	EA	T	T1
Orphan	1		777307777767771	EA	Manu3	Family33
Orphan	1		777347377413661	IO	EAI	EAI5
Orphan	1		777377777760771	EA	T	T1
Orphan	1		777700037760771	EA	T3-ETH	H37Rv
Orphan	1		777735777760731	EA	T2	T1
Orphan	1		777737477760771	EA	T	H37Rv
Orphan	1		777737757760771	EA	T3	T1
Orphan	1		777747777762661	EA	Manu2	Family33
Orphan	1		777767777420771	EA	H3-Ural-1	Haarlem3
Orphan	2		777775777760761	EA	T	T1
Orphan	1		777777637763771	EA	Manu2	Family33
Orphan	1		777777647760771	EA	T	T1
Orphan	1		777777777760771	EA	T	T1
Orphan	1		777000377740640	EA	T3-ETH	T3

TABLE 4. Drug resistance patterns to first-line antituberculosis drugs among 64 culture-positive *Mycobacterium tuberculosis* isolates in southeastern Ethiopia

Resistance	Frequency	Percentage (%)
Any drug resistance	11	17.2
STR	9	14.1
INH	7	10.9
RIF	2	3.1
EMB	0	0
Mono-resistance	6	9.4
STR only	4	6.2
INH only	2	3.1
RIF only	0	0
EMB only	0	0
Two or more drug resistance		
INH + RIF	2	3.1
INH + STR	5	7.8
INH + RIF + STR	2	3.1

Abbreviations: EMB, ethambutol; INH, isoniazid; RIF, rifampicin; STR; streptomycin.

TABLE 5. Mutations associated with rifampicin and isoniazid drug-resistant tuberculosis with Genotype MTBDRplus VER 2.0

Gene	No. of strains	Mutation pattern	Amino acid change	Result
rpoB	1 (1.1%)	ΔWT8, MUT3	S531L	RIF r
katG	5 (5.4%)	ΔWT, MUT1	S315T1	INH r

Abbreviations: Δ, deletion; INH, isoniazid; INH r, isoniazid resistant; MUT, mutant; RIF, rifampicin; RIF r, rifampicin resistant; WT, wild-type.

(5.4%) were isoniazid mono-resistant and a single strain was resistant to rifampicin. In our current sample, no isolate was resistant to isoniazid and rifampicin, i.e. no MDR isolate was detected.

The specific mutation in the five isoniazid-resistant isolates was observed at codon S315T1 of the *katG* gene, whereas mutations in the promoter region of the *inhA* gene were not observed. We found a single rifampicin-resistant isolate at Codon S531L of the *rpoB* gene, missing WT8 with the corresponding appearance of MUT3 (Table 5).

Association of lineage with drug resistance

We observed the relationship between *M. tuberculosis* strains and their drug susceptibility patterns against drug susceptibility tests for the four first-line anti-TB drugs. In our study, the BACTEC MGIT 960 drug susceptibility tests and MTBDRplus 2.0 drug susceptibility analysis identified 14 *M. tuberculosis* isolates resistant for at least one of the four first-line anti-TB drugs. All the *M. tuberculosis* strains developing resistance

belonged to the dominant Ethiopian lineage—Euro-American—and a significant rate of orphan strain resistance was observed to streptomycin.

Discussion

In the present study, 92 *M. tuberculosis* isolates from individuals with TB in the Arsi Zone were typed using RD9-based PCR and spoligotyping. The isolates were also tested for their drug sensitivity against the first-line anti-TB drugs using the MGIT 960™ system and GenoType MTBDRplus line probe assay.

The RD9-based PCR revealed that all the isolates were *M. tuberculosis*. This observation is consistent with the previous studies, which reported *M. tuberculosis* as the main cause for pulmonary TB in most parts of Ethiopia and the rest of the world [17–19]. Consistently, Nigeria reported 85% of TB was caused by *M. tuberculosis* [20]. Further strain characterization of these 92 *M. tuberculosis* isolates by spoligotyping led to the identification of 56 distinct patterns with 61% genotype diversity. Overall, 51% of the isolates were clustered into 11 different spoligotype patterns, whereas the remaining 49% of isolates were singletons. Comparable rates of clustering were found and reported in different regions of Ethiopia [21,22], South Africa (45%) [23] and in Zambia (37.7%) [24]. This high level of clustering strains suggests the presence of recent human-to-human transmission in the southeastern parts of Ethiopia. The most frequently occurring clustered strains were SIT149 (13.0%) and SIT53 (11.9%). These strains were also the most frequently circulating strains in most parts of Ethiopia [19,25,26].

Most Ethiopian TB isolates belong to three *M. tuberculosis* lineages: lineage 1 (Indo-Oceanic), lineage 3 (East-African-Indian) and lineage 4 (Euro-American lineage) [11]. The Indo-Oceanic lineage, East-African-Indian lineage, *Mycobacterium africanum* lineage, and the Euro-American lineage were also identified in the present study. Among the lineages identified, lineage 4 (Euro-American lineage) was the predominant lineage comprising 79.3% of the isolates. In agreement with our finding, the Euro-American lineage was found to be the most prevalent and widespread lineage in Ethiopia [8,11,27].This lineage is reported to be prevalent in the central highlands of Ethiopia [25,28]. Lineage 4 (the Euro-American lineage) was also the most dominant lineage in Sudanese pulmonary TB isolates (71.6%) [29]. The wide occurrence of the Euro-American lineage could be a result of its virulence and population movement between neighbouring geographic regions, which facilitate its transmission.

The second most dominant lineage identified in the present study was the East-African-Indian lineage. In line with our finding, Bedewi et al. [28] and Nuru et al. [30] reported the East-African-Indian lineage as the second most common cause of TB in the Oromia Region and in the Amhara Region of Ethiopia, respectively. With regard to grouping into families, the majority (45.7%) of the strains were members of the T1 family, while 13.1% of isolates were grouped in the T3 family, which is consistent with the findings from the Afar Region and Amhara Region [31,32].

In addition to molecular typing, the 64 liquid cultures confirmed as *M. tuberculosis* isolates were tested for sensitivity to the four first-line anti-TB drugs using MGIT 960 phenotypic drug susceptibility tests. Accordingly, we found 17.2% (11/64) showed any drug resistance. Similar proportions of resistance were reported from the Hitossa district of the Arsi Zone [13], Jimma [33] and elsewhere in Uganda [34]. However, in contrast to this present study, a higher percentage of any drug resistance was reported in Nepal [35] and in Myanmar [36].

In the present study, the GenoType MTBDRplus assay demonstrated a mutation for 5.4% (5/92) isoniazid-resistant isolates and only 1.1% rifampicin-resistant isolates. Many research findings indicate that many of the isoniazid-resistant strains contain mutations in codon 315 of *katG* [37]. In agreement with the previous reports, our finding showed that all mutations for isoniazid resistance were found in the *katG* S315T1 (5.4%) whereas no mutations were found in the promoter region of the *inhA* gene. On the other hand, mutations in the *rpoB* gene conferring rifampicin resistance were present in only 1.1% (1/92) of isolates. A similar trend for rifampicin resistance in the *rpoB* gene S531L was found by Bedewi et al. [38]. In this study, the Euro-American lineage was associated with resistance to the first-line anti-TB drugs, which agreed with the earlier reports in Ethiopia [28,39] and in Papua New Guinea [40].

Conclusion

The proportion of orphan strains isolated in this study was high, which could suggest the presence of new strains in the Arsi Zone. Moreover, the study showed a relatively high percentage of mono-resistance to any of the four first-line drugs, warranting the need to strengthen control efforts.

Conflicts of interest

The authors declare that they have no competing interests.

Authors' contributions

All the authors participated in the study design. BH contributed to conception of research idea, study design, laboratory work, analysis and interpretation of result, drafting and reviewing the manuscript and GA contributed to conception of research idea, study design, supervision, interpretation and drafting of manuscript. AZ contributed to data collection, laboratory analysis and result interpretation; BY contributed to laboratory analysis and result interpretation; and KT contributed to conception of research idea, study design, data collection, laboratory work, analysis and interpretation of result, drafting and reviewing the manuscript. GA and KT contributed equally. All authors read and approved the final manuscript.

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