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Drug Resistance Conferring Mutation and Genetic Diversity of *Mycobacterium tuberculosis* Isolates in Tuberculosis Lymphadenitis Patients; Ethiopia

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Background: Tuberculosis lymphadenitis (TBLN) is a growing public health concern in Ethiopia. However, there is limited information available on gene mutations conferring drug resistance and genetic diversity of *M. tuberculosis* isolates from TBLN patients.

Methods: Drug resistance and genetic diversity analysis were done on 91 *M. tuberculosis isolates* from culture positive TBLN patients collected between 2016 and 2017. Detection of mutations conferring resistance was carried out using GenoType MTBDRplus VER 2.0. Thereafter, isolates were typed using spoligotyping.

Results: Out of the 91 strains, mutations conferring resistance to rifampicin (RIF) and isoniazid (INH) were observed in two (2.2%) and six (6.6%) isolates, respectively. The two RIF resistant isolates displayed a mutation at codon 531 in the *rpoB* gene with amino acid change of S531L. Among the six INH resistant strains, four isolates had shown mutation at the *KatG* gene at codon 315 with amino acid change of S315T, one isolate had a mutation at the *inhA* gene at codon 15 with amino acid change of C15T and one isolate had a mutation at the *inhA* gene with unknown amino acid change. All drug resistant isolates were from treatment naive TBLN patients. The dominantly identified Spoligo International Types (SITs) were SIT25, SIT149, and SIT53, respectively; these accounted for 43% of the total number of strains. The isolates were grouped into four main lineages; Lineage 1 (2, 2.2%), Lineage 3 (38, 41.7%), Lineage 4 (49, 53.8%) and Lineage 7 (2, 2.2%). Four out of six (66.7%) isolates with drug resistance conferring mutations belonged to clustered strains (strains with shared SIT).

Conclusion: The detection of drug resistant conferring mutation in treatment naïve TBLN patients together with detection of drug resistant isolates among clustered strains might suggest resistant strains' transmission in the community. This needs to be carefully considered to prevent the spread of drug resistant clones in the country.

Keywords: drug resistant, genetic diversity, mutation, tuberculosis lymphadenitis

Introduction

Tuberculosis (TB) continues to be one of the most important public health problems, causing high morbidity and mortality, primarily in low- and middle-income countries.[1] Globally the total TB incidence has declined by an average of 1.6% per year since 2000.[1] However, the reduction in the number of extrapulmonary TB (EPTB) cases has been slower, resulting in a proportionate increase in EPTB compared to pulmonary TB (PTB).[2] EPTB represented 30% of all case of TB notified in Ethiopia, which is greater than the global average of 16%.[1] TB lymphadenitis (TBLN) accounted for 80% of all EPTB cases reported in Ethiopia.[3]

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Multidrug resistant (MDR)-TB, which is defined as being resistant at list for rifampicin (RIF) and isoniazid (INH), remains a public health problem in many parts of the world.[1] Globally, half a million people developed MDR/RIF resistant (RIF^R)-TB in 2019.[1] Ethiopia is one of 14 countries included in all three World Health Organization (WHO) high burden country lists for TB, TB/HIV, and MDR-TB.[1] Together with an increasing number of drug resistant TBs around the world, the number of cases of primary MDR-TB with EPTB presentation is also going to rise.[4] However, drug resistant EPTB is largely neglected and it does not receive specific attention in international control strategies.[5] As a result, drug resistant isolates from EPTB are not well investigated, particularly in low-income countries.

The causative agents of TB are species of the *Mycobacterium tuberculosis complex* (MTBC) comprising of seven human adopted lineages (Lineage 1–7) which show biogeographic specificities in that the individual lineages are associated with particular geographic locations.[6] Also, the MTBC includes species that are more commonly found in animals, but with zoonotic ability.[7] Lineages 2, 3 and 4 are referred to as "modern" lineages, whereas Lineages 1, 5 and 6 are called "ancient". Lineage 7 is phylogenetically localized between ancient and modern lineages and considered as a premodern lineage.[6,8,9] Although both modern and ancient lineages exist in Ethiopia, the modern lineages, particularly 4 and 3, are the most prevalent types.[10,11]

Mycobacterium tuberculosis (*M. tuberculosis)* is described as a clonal bacterium, with no known plasmid and does not engage in horizontal gene transfer. Consequently, drug resistance in *M. tuberculosis* is usually mediated by chromosomal mutations and rearrangements.[12,13] Molecular studies identified *katG* and *rpoB* as major targets conferring resistance of *M. tuberculosis* to INH and RIF respectively. Also, mutations in the regulatory region of the *inhA* operon, encoding a putative enzyme involved in mycolic acid biosynthesis, causes overexpression of the *InhA* protein, leading to INH resistance through a titration mechanism.[14,15]

Despite low genetic diversity in *M. tuberculosis* compared to other bacteria, the strain genetic background has been reported to plays a role in the global emergence of drug resistant TB. For instance, Beijing strains that belong to Lineage 2 have been frequently associated with drug resistance.[16,17] Therefore, describing drug resistance conferring mutations in *M. tuberculosis* and their strain diversity circulating in a specific geographical area is important for both biological and epidemiological reasons. Although several studies conducted in Ethiopia assessed drug resistance patterns and genetic diversity of *M. tuberculosis* isolates from PTB patients, only limited data is available in the same regard from TBLN patients. With this background, this study aims to evaluate gene mutations conferring drug resistance and to further investigate variations among *M. tuberculosis* strains of TBLN patients.

Methods

Study Setting

This study was conducted using *M. tuberculosis* isolates retrieved from Armauer Hansen Research Institute (AHRI) laboratory biorepository that have been collected between 2016 and 2017 from Bishoftu, Gondar, Mekele and Hawassa in Ethiopia, as part of the Ethiopia Control of Bovine Tuberculosis (ETHICOBOT) study. Isolates were retrieved from culture positive Fine Need Aspirate (FNA) specimens collected from TBLN patients using a convenient sampling method. Clinical and demographic information for each isolate was retrieved from the ETHICOBOT study database using structured data extraction sheets.

Drug Resistance Testing Using GenoType MTBDRplus VER 2.0

RIF and INH resistance conferring mutation was detected using GenoType MTBDRplus VER 2.0. (Hain Life Science GmbH, Nehren, Germany) according to the manufacturer's instruction. The test is based on DNA strip technology and has three steps: DNA extraction, amplification, and reverse hybridization. GenoType MTBDRplus VER 2.0 detects the absence and/or presence of wild type (WT) and/or mutant (MUT) DNA sequences within specific regions of three genes: the *rpoB* gene-gene (coding for the β-subunit of the RNA polymerase), for the identification of RIF^R; the *katG* gene (coding for the catalase peroxidase), for high level INH resistance (INH^R); and the promoter region of the *inhA* gene (coding for the NADH enoyl ACP reductase), for low level INH^R. GenoType MTBDRplus included eight *rpoB* WT probes, four *rpoB* MUT probes in positions of *rpoB* MUT1 (D516V), *rpoB* MUT2A (H526Y), *rpoB* MUT2B (H526D) and *rpoB* MUT3 (S531L), one *katG* WT probe, two *katG* MUT probes with *katG* MUT1 (S315T1) and *katG* MUT2 (S315T2), two *inhA* WT probes and four *inhA* MUT

probes with *inhA* MUT1 (C15T), *inhA* MUT2 (A16G), *inhA* MUT3A (T8C) and inhA MUT3B (T8A). According to the manufacturer's recommendations, the missing of a WT probe or presence of a MUT probe were considered as resistant.

Spoligotyping

Spoligotyping was carried out as described by Kamerbeek et al.[18] Spoligotype patterns of each strain were prepared in binary and octal format and entered into spoligotyping database SITVIT2 (http://www.pasteur-guadeloupe.fr:8081/SITVIT2/), which is an updated version of SITVITWEB.[19] Strains matching a preexisting pattern in the database were identified with Spoligo International Type (SIT) number, otherwise considered as orphans or new. Run TB-Lineage online tools (http://tbinsight.cs.rpi.edu/run_tb_lineage.html) was used to predict major *M. tuberculosis* lineages.[20] Nomenclature for lineage names and numbers were assigned as proposed previously. For instance, Lineage 1 (Indo Oceanic; IO), Lineage 3 (East African-Indian; EAI), Lineage 4 (Euro-American; EA) and Lineage 7 (Ethiopian).[21,22]

Data Quality Assurance

Standard operational procedures for all laboratory tests were employed uniformly throughout the study. PCR was carried out in three separate rooms for DNA extraction, PCR mix preparation and amplification using dedicated pipettes and sterile tips. Furthermore, DNA from *M. bovis* Bacille Calmette-Guerin and *M. tuberculosis* H37Rv were used as positive controls while DNA free water from Qiagen was used as a negative control in each batch of the test.

Data Analysis

Data were double entered to an Excel file format and statistical analysis was performed using SPSS version 20 (IBM Corp, Armonk, NY, USA). Descriptive statistics were used to depict the demographic variables. The Fisher exact was calculated to test the association between drug resistant conferring mutation and specific lineages of *M. tuberculosis* isolates. P-value ≤ 0.05 was considered statistically significant.

Ethical Consideration

Ethical approval was obtained from the AHRI/ALERT Ethics Review Committee. Since the entire repository data were anonymized, no personal identifiers were collected during data retrieval.

Results

Demographic Data and Isolates Information

A total of 91 *M. tuberculosis* isolates obtained from TBLN patients were included in this study, of which 54 (59.3%) were from females and 37 (40.7%) from males. The patients' mean age was 32 years with a range of 9–76 years (Table 1). Of the 91 isolates included in this study, 35 (38.5%), 27 (29.7%), 21 (23.1%) and 8 (8.8%) were collected from Bishoftu, Gondar, Mekele and Hawassa, respectively.

Drug Resistant Conferring Mutation in *Mycobacterium tuberculosis* Isolates

Of 91 isolates tested for GenoType MTBDRplus VER 2.0, mutations conferring resistance to RIF and INH were observed in two (2.2%) and six (6.6%) isolates, respectively. Two (2.2%) of them were MDR isolates. Of isolates with resistant mutations, two (2.2%) were in the *rpoB* gene, four (4.4%) were in the *katG* gene and two (2.3%) were in the *inhA* promoter region. In two RIF^R isolates, mutation was observed at codon S531L indicated by missing of *rpoB* WT8 probe with gain in *rpoB* MUT3 probes. Four of the six INH^R isolates had a *katG* mutation. In three of these isolates, the mutation was observed at codon S315T1 indicated by absence of *KatG* WT with gain in *katG* MUT1 whereas one isolate had a mutation at S315T2 indicated by the absence of *KatG* WT with gain of *KatG* MUT2. Mutations in the *inhA* promoter gene occurred in two INH^R isolates. One of these isolates had a mutation at codon C15T which was indicated

Table 1 Demographic Characteristics of Study Participants from Different Places in Ethiopia, 2016–2017

Variables	Frequency	Number
Sex		
Male	37	40.7%
Female	54	59.3%
Age Group		
9–21	30	32.9%
22–31	20	21.9%
32–41	6	6.6%
42–51	21	23.1%
>51	14	15.4%
Patient Category		
New Case	71	78%
Retreatment	17	18.7%
Unknown	3	3.3%

by the omission of *inhA* WT1 and with the presence of the *inhA* MUT1 band. In one isolate *inhA* MUT1 band developed without missing the WT probe (Table 2). All drug resistant isolates were from treatment naive TBLN patients.

Genetic Diversity in *Mycobacterium tuberculosis* Isolates

Among the 91 spoliogotyped isolates, 82 (90.1%) were classified into 28 different spoligotyping patterns according to the SITVIT2 database. The remaining 9 (9.9%) isolates were not registered in the database and thereby seen as new or orphans' strains. The dominating identified SITs were SIT25, SIT149 and SIT53, each consisting of 19 (20.9%), 11 (12.1%) and 9 (9.9%) isolates, respectively (Figure 1).

Lineage 3 (EAI) was the most prevalent lineage in Gondar (18/27, 66.7%) and Mekele (11/21, 52.3%), whereas Lineage 4 (EA) was the most prevalent lineage in Bishoftu (27/35, 77.1%) and Hawassa (7/8, 87.5%). Two strains belonging to Lineage 1 (IO) were isolated in this study, both of them were from Mekele (SIT726). Furthermore, two Lineage 7 (Ethiopian) isolates were identified, one from Mekele (SIT910) and one from Gondar (SIT1729). Overall, Lineage 3 and Lineage 4 were the most prevalent lineages identified in this study, each accounted for 41.7% (38/91) and 53.8% (49/91), respectively. Whereas Lineage 1 and Lineage 7 were the least prevalent lineages, each accounted for 2.2% of the total isolates (Table 3).

Cluster analysis based on spoligotyping patterns showed that 63 isolates were grouped into 9 clusters; as one cluster consisted of 2–19 isolates. The clustering rate was 69.2%. Statistically significant different rate of clustering observed between major MTBC lineages (Fisher's Exact test = 8.413; P = 0.017) (Table 4).

Drug Resistance Conferring Mutation and *Mycobacterium tuberculosis* Lineages

Isolates with drug resistance conferring mutations for any of the anti-TB drugs (RIF or INH), tested for by GenoType MTBDRplus, belonged to Lineage 3 (50%; 3/6) and Lineage 4 (50%; 3/6). However, an association between having anti-TB drug conferring mutation and major *M. tuberculosis* lineages were not statistically significant (Fisher exact test: 1.355; $p > 0.05$). Four out of six (66.7%) of the drug resistant isolates in this study belonged to a clustered strain (strains with shared SIT). Out of the three resistant strains of Lineage 3, one MDR-TB isolate with *rpoB* and *KatG* mutations was of SIT25, and two INH^R isolates with *inhA* mutation had SIT26 and Orphan spoligotypes, whereas, among the resistant strains of Lineage 4, one MDR-TB isolate with *rpoB* and *KatG* mutations was of SIT149, two INH^R isolates with a *katG* mutation were of SIT 50 and SIT 149 (Table 2).

Discussion

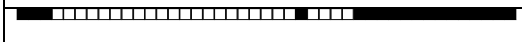
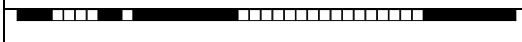
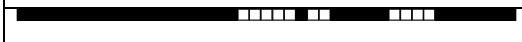
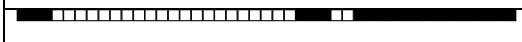
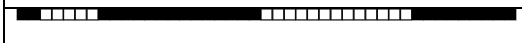
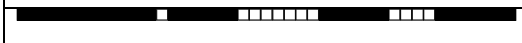
This study presented the magnitude of drug resistance conferring mutation and genetic diversity of *M. tuberculosis* strains that cause TBLN in Ethiopia. Among the 91 isolates included in this study, mutations conferring resistance to RIF, INH, and to both of these drugs (MDR-TB), were observed in 2 (2.2%), 6 (6.6%), and 2 (2.2%) isolates, respectively. 2.2% MDR prevalence in this study was comparable with previous studies reported from Ethiopia among TBLN (1–4%)[23–25] and PTB patients (1–3%).[25–27]. The problem of MDR in TBLN patients should not be ignored and early diagnosis of drug resistance is crucial to avoid the devastating effect of MDR TB.

The two RIF^R isolates identified in this study contained the S531L mutation in the *rpoB* gene, which is the most frequently reported *rpoB* mutation in the Ethiopian strains,[23,28–30] indicating the possible transmission of strains with similar types of mutations in the community. However,

Table 2 Mutations Identified in Isoniazid and Rifampicin Resistant *M. tuberculosis* Strains

Anti-TB Drugs	Gene	Pattern of Gene Mutations (WT/MUT)	Amino Acid Change	Resistance Pattern	SIT
Rifampicin	*rpoB*	ΔWT8/MUT3	S531L	RIF^R	SIT149, SIT25
Isoniazid	*KatG*	ΔWT/MUT1 ΔWT/MUT2	S315T1 S315T2	INH^R INH^R	SIT149, SIT25, SIT50 SIT149
	inhA	ΔWT1/MUT1 ND/MUT1	C15T unknown	INH^R INH^R	SIT26 Orphan

Note: SIT149 and SIT25 were MDR strains (resistant to INH and RIF).
Abbreviations: Δ, deletion; WT, wild type; MUT, mutant; ND, no mutation detected at wildtype probe; RIF^R, rifampicin resistant; INH^R, Isoniazid resistant; SIT, spoligotype international types.

Binary Format (presence (black) or absence (white) of 43 spacers)	Octal Code	SIT	Major Lineage	N (%)
	703777740003171	25	EAI (Lineage 3)	19 (20.9)
	777000377760771	149	EA (Lineage 4)	11 (12.1)
	777777777760771	53	EA (Lineage 4)	9 (9.9)
	703777740003771	26	EAI (Lineage 3)	8 (8.8)
	777737777760771	37	EA (Lineage 4)	6 (6.6)
	703677740003171	2359	EAI (Lineage 3)	4 (4.4)
	777777777760731	52	EA (Lineage 4)	2 (2.2)
	777777777763771	54	EA (Lineage 4)	2 (2.2)
	777737747413771	726	IO (Lineage 1)	2 (2.2)
	777777770020771	47	EA (Lineage 4)	1 (1.1)
	777777777720771	50	EA (Lineage 4)	1 (1.1)
	777737777420731	1134	EA (Lineage 4)	1 (1.1)
	703777740000000	1264	EAI (Lineage 3)	1 (1.1)
	700000004177771	1729	AFRI (Lineage7)	1 (1.1)
	703377400001771	21	EAI (Lineage 3)	1 (1.1)
	703701740003171	2973	EAI (Lineage 3)	1 (1.1)
	777737377720771	3134	EA (Lineage 4)	1(1.1)
	776737777760771	3137	EA (Lineage 4)	1 (1.1)
	177000377760771	3141	EA (Lineage 4)	1 (1.1)
	777776777760731	336	EA (Lineage 4)	1 (1.1)
	777777404760771	41	EA (Lineage 4)	1 (1.1)
	777777607760771	42	EA (Lineage 4)	1 (1.1)
	777737770000000	56	EA (Lineage 4)	1 (1.1)
	677777777720571	699	EA (Lineage 4)	1 (1.1)
	777757777720771	764	EA (Lineage 4)	1 (1.1)
	777777777420731	817	EA (Lineage 4)	1 (1.1)
	700000007177771	910	AFRI (Lineage 7)	1 (1.1)
	603777740003771	952	EAI (Lineage 3)	1 (1.1)
	777000277760771	Orphan or New	EA (Lineage 4)	1 (1.1)
	603777700003771	Orphan or New	EAI (Lineage 3)	1 (1.1)
	503777740003171	Orphan or New	EAI (Lineage 3)	1 (1.1)
	777737401760771	Orphan or New	EA (Lineage 4)	1 (1.1)
	777737777760000	Orphan or New	EA (Lineage 4)	1 (1.1)
	403000377760771	Orphan or New	EA (Lineage 4)	1 (1.1)
	703777700001171	Orphan or New	EAI (Lineage 3)	1 (1.1)
	776737737760771	Orphan or New	EA (Lineage 4)	1 (1.1)
	777777777420571	Orphan or New	EA (Lineage 4)	1 (1.1)

Key: SIT, Spoligo International Type; EA, Euro-American, IO; Indo-Oceanic; EAI, Euro American Indian; N, Number

Figure 1 Spoligotypes and major lineage classifications of clinical *M. tuberculosis* strains isolated from TBLN patients in different places in Ethiopia, 2016–2017.

mutations at other codons including H526D and D516V had also been reported among RIF^R isolates.[28–31] Both RIF^R strains in this study were INH^R. Mono resistance to RIF is quite rare and almost all RIF^R strains were also resistant to other drugs, especially to INH, which is why RIF^R is considered as a surrogate marker for MDR-TB.[15]

Table 3 *M. tuberculosis* Lineage Distribution in Different Sample Collection Places in Ethiopia, 2016–2017

Collection Site	Total Number of Isolates	L1 N/%	L3 N/%	L4 N/%	L7 N/%
Bishoftu	35	0	8/22.8	27/77.1	0
Gondar	27	0	18/66.7	8/29.6	1/3.7
Mekele	21	2/9.5	11/52.3	7/33.3	1/4.8
Hawassa	8	0	1/12.5	7/87.5	0
Total no. of isolates/%	91/100	2/2.2	38/41.7	49/53.8	2/2.2

Abbreviations: N, number; L, lineage.

Resistance to INH is frequently associated with a mutation at two genes; *katG* and *inhA*. In this study, 67% (4/6) of INH^R isolates had a *katG* gene mutation at codon S315T. In contrast to this, 100% frequency of *KatG* mutation at codon S315T among INH^R isolates had been reported from Ethiopia.[23,28,30,32] Moreover, in the current study, gene mutations attributed to low level INH^R mainly caused by the mutations in the promoter region of *inhA* gene were also observed. Such mutations were more frequent in our study (43%, 3/7) than the 10–12% reported by other studies conducted in Ethiopia,[29,33] in Pakistan (17%),[34] and in Switzerland (23%).[35] Mutations in *inh*A gene not only causes resistance to INH but also to the structurally related drug ethionamide, which shares the same target.[14] Of the two isolates with *inhA* mutation, one of them had a mutation at C15T, whereas the other one had *inhA*MUT1 without mutation on the corresponding WT probe indicating heteroresistant isolates, ie concomitant infection with drug-resistant and drug-susceptible strains. TB infection with a heteroresistant *M. tuberculosis* population can be caused by infection with two different strains or the splitting of a single strain into susceptible and resistant organisms through microevolution.[36,37] The relevance of heteroresistant TB should not be underestimated especially in highly endemic areas like Ethiopia, where there is a chance of co-infection with different *M. tuberculosis* strains with different resistant conferring mutations.

The *M. tuberculosis* population structure in this study was highly diverse and comprised of 28 different SITs and nine orphans or new strains. It is not unexpected, considering the samples are collected from different regions of the country. Lineage 3 and Lineage 4 were the most prevalent lineages identified in this study, each accounted for 41.7% and 53.8%, respectively. Similar patterns of lineage distribution were reported from different regions of Ethiopia among EPTB[38–41] and PTB patients.[26,30] Likewise, a study that analyzed the distribution of genotypes among PTB and TBLN patients in Ethiopia, reported a similar distribution of genotypes between the two manifestations of the disease.[8] This may indicate the absence of pathogen-specific genetic factors associated with the high rate of TBLN in Ethiopia and also suggested a similar route of PTB and TBLN transmission in the community. Lineage 4 has a broad distribution in Europe and America, Africa and the Middle-East whereas Lineage 3 has a relatively narrow distribution occurring in East Africa and Central and South Asia.[9]

Table 4 Cluster Distribution Among Different *Mycobacterium tuberculosis* Lineages

Lineage	Total Isolate	Cluster Strains	Cluster %	Cluster Number	Cluster Size	SIT Number
L1	2	2	100%	1	2	SIT 726
L3	38	31	81.5%	3	19 8 4	SIT 25 SIT 26 SIT 2339
L4	49	30	61.2%	4	11 9 6 2 2	SIT 149 SIT 53 SIT 37 SIT 54 SIT 52

Abbreviations: AHRI, Armauer Hansen Research Institute; EPTB, extrapulmonary tuberculosis; ETHICOBOTs, Ethiopia Control of Bovine Tuberculosis; INH, isoniazid; INH^R, isoniazid resistant; MDR, multidrug resistant; MTBC, *Mycobacterium tuberculosis* complex; MUT, mutant; PTB, pulmonary tuberculosis; RIF, rifampicin; RIR^R, rifampicin resistant; TB, tuberculosis; TBLN, tuberculosis lymphadenitis; SIT, Spoligo International Type; WHO, World Health Organization, WT; wild type.

Lineage 1 and Lineage 7 were the least prevalent lineages in this study, each accounted for 2.2% of the 91 TBLN isolates, which is in line with the overall relatively low prevalence of these lineages in Ethiopia.[10,11] Lineage 1 is found in areas around the Indian Ocean and the Philippines.[9] The Lineage 1 isolates (two isolates) in this study were isolated from Mekele TBLN patients. Previously, Lineage 1 was also reported from Southern,[8,42] Central[39] and North Ethiopia.[32,43] The two Lineage 7 isolates identified in this study were isolated from Mekele and Gondar TBLN patients. This is the newly identified lineage initially reported in higher frequency from Woldia in Northern Ethiopia.[8] Since then, it has been reported from different regions of the country.[38,40,44,45] Lineage 7 has also been reported among Ethiopian immigrants in Djibouti and the Netherlands.[46,47] The reason why Lineage 7 is restricted to native Ethiopians and Ethiopian immigrants is not yet well understood but it has been indicated that Lineage 7 has a lower rate of progression towards disease relative to other lineages, with subsequent out competition by other *M. tuberculosis* lineages.[44] That may explain the geographical restriction of Lineage 7 to Ethiopia. Lineage 7 has contributed to the rejection of the "virgin soil" hypothesis of human TB in sub-Saharan Africa. According to "virgin soil" hypothesis, TB in the African region was due to European contact during the colonial period as it was originally free of TB.[48]

In our study, the overall clustering rate (strains with shared SIT) was 65.6% which is in line with other studies conducted in Ethiopia.[8,26,41,49] A high rate of clustering maybe indicates active transmission of the disease and an ineffective TB control programme in the country. However, the low discrimination power of spoligotyping should be considered.[50]

The majority (4/6, 66.7%) of isolates with drug resistant conferring mutations in this study belonged to clustered strains, suggesting the possibility of transmission of drug resistant isolates between patients in the country. Moreover, all drug resistant isolates identified in the current study were from treatment of naïve TBLN patients. That supports exposure of the patients to drug resistant *M. tuberculosis* strains in the community, rather than susceptible strains becoming resistant during the TB treatments. This needs to be carefully considered to prevent the spread of drug resistant clones in the country. Of the six isolates with drug resistant conferring mutations, two (33%) of them were SIT 149 whereas the rest were SIT 25, SIT 26, SIT 50 and one orphan strain. The high frequency of SIT149 among drug resistant *M. tuberculosis* isolates has been previously reported in Ethiopia.[51,52] However, Bereket et al indicated that the observed association between SIT149 and the development of drug resistance may not necessarily indicate that the stains are prone to be drug resistant but could rather be association consequences of their high prevalence in the population.[25]

No significant associations were found between a particular lineage and any drug resistant conferring mutation. However, this might have been due to the low sample size. Apart from results shown for Lineage 2,[16,17] the association between different *M. tuberculosis* lineages and TB drug resistance is rather inconsistent. For instance, Biadglegne et al[40] and Tadesse et al[38] showed a significant association between drug resistance and Lineage 3, whereas Amir et al found an association between Lineage 4 and drug resistance conferring mutations.[32] In contrast, other studies did not find associations between the genotype of *M. tuberculosis* isolates and their drug resistance pattern.[53,54] This shows that there is uncertainty on the strain-specific propensity for the acquisition of drug resistance conferring mutation among *M. tuberculosis* isolates. More work needs to be done to define whether some *M. tuberculosis* genotypes are more prone than others to develop drug resistance.

Conclusion

Overall, although the sensitivity of the GenoType MTBDRplus assay to detect strains with a novel mutation or gene mutation outside the resistance determining region is limited, the present study demonstrated the feasibility of estimating the magnitude of gene mutations conferring drug resistance and genetic diversity of drug resistant *M. tuberculosis* isolates in TBLN patients. Lineage 3 and Lineage 4 were the most prevalent lineage types identified in this study with high clustering rates of SIT 25, SIT149 and SIT 53. A drug resistant conferring mutation was detected among clustered strains, which could suggest clonal resistant strains transmission in the community. However, the tool we used to characterize the different *M. tuberculosis* strains, spoligotyping, is prone to convergent evolution and has low resolution power for cluster analysis. This warrants the need for future studies with a better tool of discrimination power like whole-genome sequencing (WGS) to understand the transmission dynamics of drug resistant TB and strengthen the control programs of TBLN in Ethiopia.

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Disclosure

The authors report no conflicts of interest for this work.

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