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Strain diversity and gene mutations associated with presumptive multidrug-resistant *Mycobacterium tuberculosis* complex isolates in Northwest Ethiopia

Mebrat Ejo[a,b,c,*], Gabriela Torrea[a], Ermias Diro[d,e], Ayenesh Abebe[f], Meseret Kassa[f], Yilak Girma[f], Eyasu Tesfa[e], Kefialew Ejigu[g], Cecile Uwizeye[a], Florian Gehre[h,i], Bouke C. de Jong[a], Leen Rigouts[a,c]

[a] *Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium*
[b] *Department of Biomedical Sciences, University of Gondar, Gondar, Ethiopia*
[c] *Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium*
[d] *Department of Internal Medicine, University of Gondar, Gondar, Ethiopia*
[e] *MDR-TB Treatment and Follow-up Center, University of Gondar Specialized Hospital, Gondar, Ethiopia*
[f] *TB culture laboratory, University of Gondar Specialized Hospital, Gondar, Ethiopia*
[g] *TB culture laboratory, Amhara Public Health Institute, Bahir Dar, Ethiopia*
[h] *Department of Infectious Disease Epidemiology, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany*
[i] *East African Community Secretariat, Arusha, Tanzania*

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ABSTRACT

Objectives: In this study, we assessed the genetic diversity and gene mutations that confer resistance to rifampicin (RIF), isoniazid (INH), fluoroquinolone (FQ), and second-line injectable (SLI) drugs in RIF-resistant (RR)/multidrug-resistant tuberculosis (MDR-TB) isolates in Northwest Ethiopia.
Methods: Spoligotyping was used to assign isolates to TB lineages (Ls), and Hain line probe assays were used to detect resistance to RIF, INH, and FQs, and SLIs.
Results: Among 130 analyzed strains, 68.5% were RR, and four major *Mycobacterium tuberculosis* complex lineages (L1, L3, L4, and L7) were identified with a predominance of the Euro-American L4 (72, 54.7%), while L7 genotypes were less common (3, 2.3%). Overall, the L4-T3-ETH (41, 32.0%), L3-CAS1-Delhi (29, 22.7%), and L3-CAS1-Killi (19, 14.8%) families were most common. Line probe analysis showed that among *rpoB* mutants, 65.2% were S450L, while 87.8% of *katG* mutants were S315T. Only three isolates showed mutation (c-15t) at the *inhA* gene, and no double mutation with *katG* and *inhA* genes was found. Six strains, two each of L1, L3, and L4, were resistant to FQs, having *gyrA* mutations (D94G, S91P), of which three isolates had additional resistance to SLI (*rrs* A1401G or C1402T mutations) including one isolate with low-level kanamycin (KAN) resistance.
Conclusions: This study showed a predominance of L4-T3-ETH, L3-CAS1-Delhi, and L3-CAS1-Killi families, with a high rate of *rpoB*_S450L and *katG*_S315T mutations and a low proportion of *gyrA* and *rrs* mutations. L7 was less frequently observed in this study. Further investigations are, therefore, needed to understand L7 and other lineages with undefined mutations.

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1. Introduction

Despite evidence that tuberculosis (TB) is slowly declining [1], the emergence and spread of rifampicin- and multidrug-resistant (RR/MDR) strains of *Mycobacterium tuberculosis* complex (MTBC) is a major public health concern and threatens the global control of the disease [1,2]. In 2019, an estimated 3.3% of new cases and 18% of previously treated cases had RR/MDR-TB worldwide [1]. In Ethiopia, one of the high TB/HIV and RR/MDR-TB burden countries, the total TB incidence is 151 per 100 000 population, with RR/MDR-TB prevalence ranging from 0.71% in newly notified patients to 12% of previously treated patients, and an

* Corresponding author. Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium.
E-mail address: drmebrat@yahoo.com (M. Ejo).

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estimated 1400 RR/MDR-TB cases reported annually [1]. The presence of RR/MDR-TB strains has been reported as an emerging problem [3]. Since 2014, the country has implemented the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) in 285 referral health facilities for the diagnosis of TB and detection of RR-TB [4]. At present, Ethiopia prioritizes early TB case detection with enhanced capacity to diagnose RR/MDR-TB and provision of appropriate treatment, which together prevent further transmission of TB/MDR-TB in the community. Nevertheless, the available studies of RR/MDR-TB MTBC strains are limited because of incomplete drug-resistant TB surveillance and the lack of the required diagnostic facilities in the country.

The prevalence of drug-resistant TB varies geographically [1], with rapid increases in the incidence of MDR-TB in resource-poor countries. In Ethiopia, the main reasons contributing to this increase remain elusive; however, the genetic differences among the mycobacterial strain lineages may contribute to resistance-conferring mutation [5,6] together with population crowding, HIV/AIDS epidemics, and poor treatment adherence [7].

MTBC strain genetic makeup has been described elsewhere to play a role in the emergence of drug resistance [8]. Variations between lineages of MTBC have been shown, with the modern lineages and families being more frequently associated with drug resistance [9]. While MTBC has a relatively low mutation rate, over the long period of co-evolution with its host, it has accumulated differences that affect the presentation and outcome of TB [10]. It has also been reported that the type of drug resistance-conferring mutation varies among diverse strains of MTBC lineages [11]. For example, the Euro-American L4 was more likely to harbor the *katG* S315T mutation, and the Indo-Oceanic L1 had the *inhA* promoter mutation [11,12]. In recent years, significant progress has been made in the understanding of the molecular basis of resistance-conferring mutations of anti-TB drug resistances in MTBC strains [11,13]. However, limited data is available in Ethiopia.

Although few molecular studies have been performed among MTBC isolates from different regions of Ethiopia [5,14], there is a paucity of data on the extent of various lineages and resistance-conferring mutations of MDR-TB isolates representative of the Amhara region in Ethiopia. Therefore, in the present study, we sought to investigate the genotype diversity, geographical distribution, and resistance-conferring mutations of MDR strains in Northwest Ethiopia by using Spoligotyping and line probe assay (LPA) methods.

2. Methods

2.1. Study setting and clinical isolates

Prospective and retrospective studies were carried out on baseline clinical isolates obtained from pulmonary RR/MDR-TB patients referred to the two TB culture laboratories at Gondar and Bahir Dar (Fig. 1). These two laboratories provide TB referral diagnostic services for genotypic and phenotypic drug-susceptibility testing to all peripheral health facilities in Northwest Ethiopia. For the prospective study, we enrolled all consecutive RR-TB patients identified by Xpert MTB/RIF admitted in the MDR-TB treatment center at the University of Gondar Hospital (between 2017–2019), while for the retrospective study we reviewed the lab registration book between 2013–2016 to find the available RR/MDR-TB isolates stored at both laboratories, allowing a single isolate per patient. Isolates were diagnosed as drug-resistant TB using Xpert MTB/RIF and LPAs. Phenotypic drug-susceptibility testing (pDST), however, was not available because of logistic reasons. Socio-demographic data such as sex, age, and body mass index (BMI) were captured.

All clinical mycobacterial isolates used in this study were obtained from a program for the diagnosis and treatment of RR/MDR-TB in the Amhara regional state, hosting a population of more than 30 million people and known as a high TB incidence area.

2.2. Mycobacterial culture and identification

The collected sputa from prospective RR-TB patients were cultured on Löwenstein-Jensen (LJ) media following the WHO-recommended standard procedure for the isolation and identification of mycobacteria [15]. Stored mycobacterial isolates were drawn from the -20°C freezer, thawed, and sub-cultured on LJ medium slants. Identification of MTBC in cultures was confirmed by acid-fast staining and the MPT64 antigen test [15]. The laboratory strain, *M. tuberculosis* H37Rv, was used as a positive control.

2.2.1. DNA extraction

Mycobacterial DNA extraction was performed using the GenoLyse® DNA extraction kit (Hain LifeScience, Nehren, Germany) according to the manufacturer's protocol [16]. DNA extracts were used directly or stored at -20°C until GenoType MTBDR*plus* and -*sl* LPAs and the spoligotyping were performed.

2.2.2. Spoligotyping

Spoligotyping was carried out following the described protocol using in-house prepared membranes [17]. The results were interpreted for the presence or absence of spacers from spoligotype images using the consensus of binary digits and an octal numbering system [17,18]. Both *M. tuberculosis* H37Rv and *M. bovis* BCG reference strains' DNAs were included as positive controls and sterilized water as a negative control in each run.

2.2.3. GenoType Line Probe Assays (LPAs)

Polymerase chain reaction-based LPAs, such as GenoType MTBDR*plus* and -*sl* Version 2.0 LPAs (Hain Life Science, Nehren, Germany) were performed according to the manufacturer's instructions [16]. The result was taped to the MTBDR*plus* and/or MTBDR*sl* assay worksheets for interpretation as described in the guidelines [19].

2.3. Spoligotype database comparison

To assign MTBC lineages and families, spoligotypes were analyzed using the newly revised publicly available international spoligotyping (SITVIT2) database of the Pasteur Institute of Guadeloupe (http://www.pasteur-guadeloupe.fr:8081/SITVIT2) [20]. Besides, the MIRU-VNTRplus database, TBlineage (http://tbinsight.cs.rpi.edu/about_tb_lineage.html), and Spotclust (http://tbinsight.cs.rpi.edu/run_spotclust.html) were used to identify MTBC families. Lineages and families were defined according to signatures provided in SpolDB4 and SITVIT2 [20,21]. A spoligotype international type (SIT) was assigned when two or more patient isolates in the database shared identical spoligotype patterns, while spoligotype patterns that had not been described before were defined as 'orphan.'

2.4. Statistical Analysis

Statistical analysis was performed using STATA 15.1 (Stata Corp, College Station, TX). Descriptive statistics such as frequency, percentage, and/or proportion were used to analyze the socio-demographic data, MTBC lineages, and genotypic LPA results. The two-sided Pearson's Chi-Square test and/or the two-sample proportion test were used to evaluate associations of drug resistance and spoligotype families. An association of anti-TB drug resistance with MTBC genotypes was expressed using a *P* value and 95% confidence interval (95% CI). A *P* value of less than 0.05 was considered statistically significant.

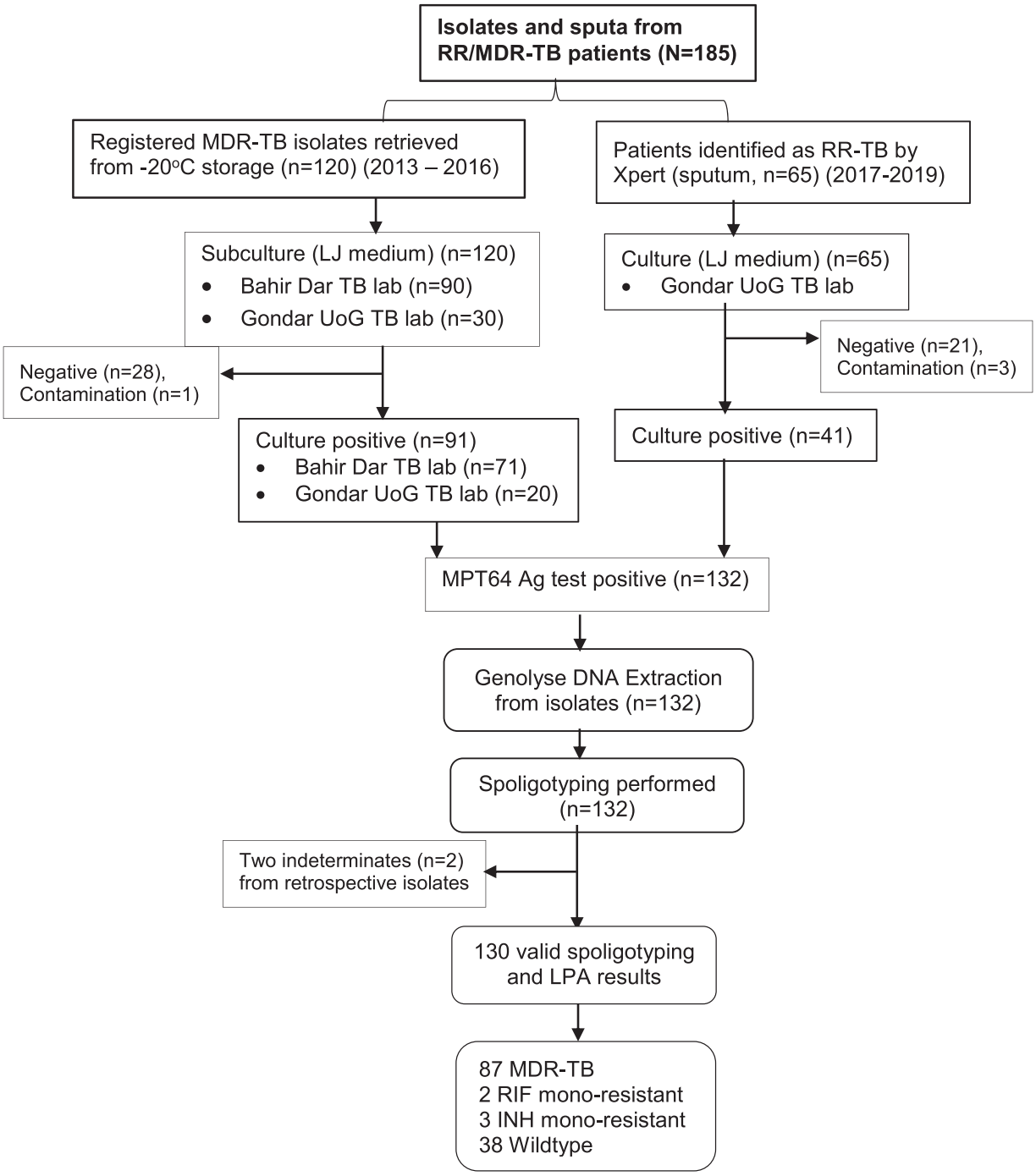

Fig. 1. Isolates from presumptive MDR-TB patients included in spoligotyping and line probe assay (LPA) analysis

3. Results

A total of 185 presumptive RR/MDR-TB patient samples were included in this study: 65 sputa and 120 retrieved stored MTBC isolates (Fig. 1). Of these, only 132 (71.4%) had growth in culture (63.1% sputa and 75.8% stored isolates).

Of the 130 RR/MDR-TB patients included in the analysis, the majority (n = 95; 73.1%; 95% CI 64.6–80.5%) were male. The median age and BMI of the patients were 32.0 (IQR = 25.0–40.0) and 18.0 (IQR = 17.0–19.0), respectively. Socio-demographic variables such as sex, age group, and BMI did not differ significantly between prospectively or retrospectively recruited patients (data not shown).

3.1. Mycobacterial lineage and family

Spoligotyping of the 130 MTBC isolates yielded 35 spoligotypes patterns, 21 previously registered in SITVIT2 [20] and 14 of these patterns were unique (1 isolate only). All spoligotyped isolates were grouped into four lineages, with the predominance of Euro-American L4 (n=72, 55.4%) and East African Indian L3 (n = 52, 40.0%), and a minority of the Indo-Oceanic L1 and Ethiopian lineage L7 (n = 3, 2.3% respectively) (Supplementary Table S1).

A total of 35 different spoligotypes were identified. Among these, 21 had been described in SITVIT2 [20], and 14 were unique. All spoligotyped isolates were classified into four MTBC lineages (Ls), with the predominance of Euro-American L4 (72, 55.4%) and

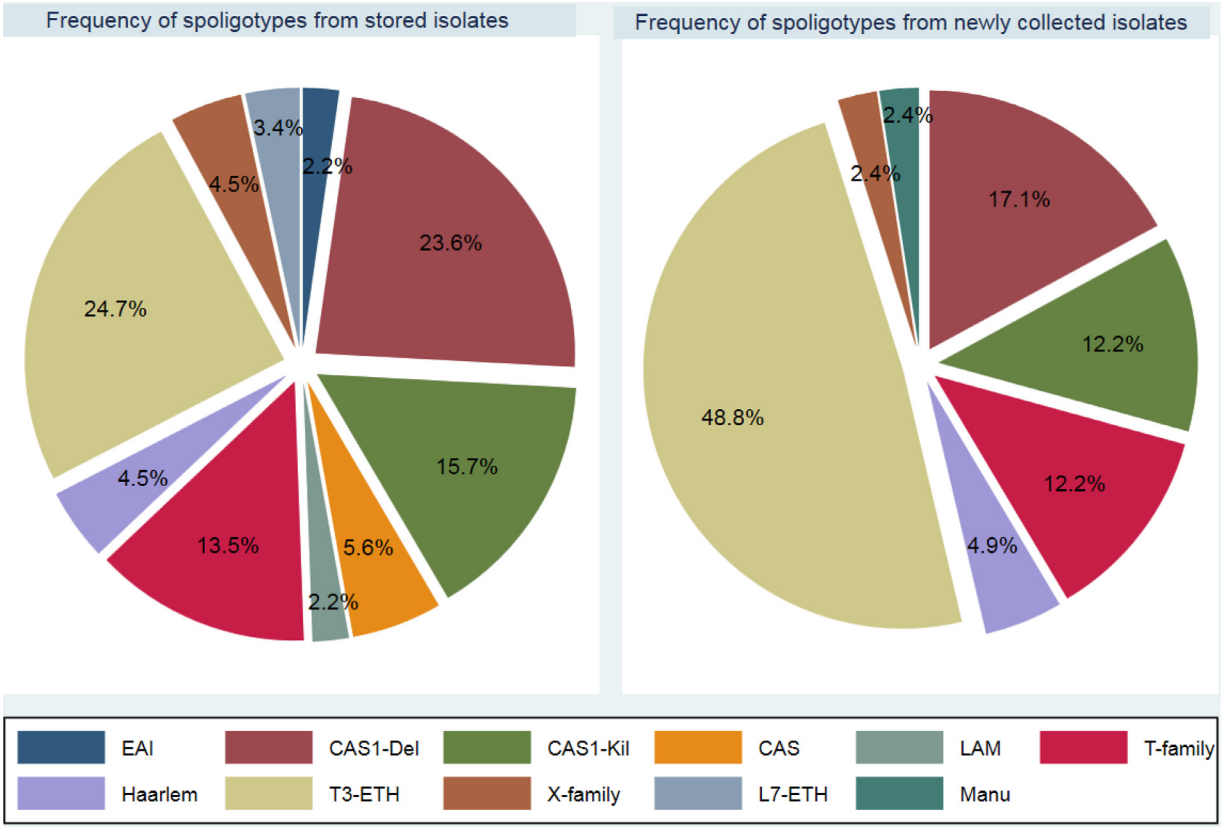

Fig. 2. *Mycobacterium tuberculosis* complex (MTBC) families from retrospectively (n = 89) and prospectively (n = 41) collected TB isolates. CAS, Central Asian; LAM family, Latin American-Mediterranean

Table 1
Mycobacterium tuberculosis complex (MTBC) lineages and families identified from RR/MDR-TB patients in Northwest Ethiopia

MTBc Lineage (L) (n)	Family	Isolates			
		Retrospective, (%)n = 89	Prospective, (%)n = 41	OR	95% CI
Indo-Oceanic (L1) (3)	Manu	0 (0.0)	1 (2.4)	-	-
	Family33	2 (2.3)	0 (0.0)	-	-
East African Indian (L3) (52)	CAS1-Delhi	21 (23.6)	7 (17.1)	1.5	0.58–3.87
	CAS1-Kili	14 (15.7)	5 (12.2)	1.34	0.45–4.02
	CAS-family	5 (5.6)	0 (0.0)	-	-
Euro-American (L4) (72)	Haarlem	4 (4.5)	2 (4.9)	0.92	0.16–5.22
	T-family	12 (13.5)	5 (12.2)	1.1	0.37–3.42
	T3-ETH	22 (24.7)	20 (48.8)	0.34	0.16–0.75
	LAM	2 (2.3)	0 (0.0)	-	-
	X-family	4 (4.5)	1 (2.4)	1.9	0.20–17.39
Ethiopian lineage (L7) (3)	ETH1- L7	3 (3.4)	0 (0.0)	-	-

CAS, Central Asian; Ci, confidence interval; L, lineage; LAM family, Latin American-Mediterranean; MTBC, *Mycobacterium tuberculosis* complex; OR, odds ratio.

East African Indian L3 (n = 52, 40.0%), and a minority of the Indo-Oceanic L1 and the Ethiopian lineage L7 (both with 3 cases, 2.3% of the total) (Supplementary Table S1).

Overall, the Ethiopian T3-ETH (22/89, 24.7%), CAS1-Delhi (21/89, 23.6%), CAS1-Killi (14/89, 15.7%), and T-family (12/89, 13.5%) were common spoligotypes found among stored isolates, while the Ethiopian T3-ETH (SIT149) (20/41, 48.8%) was the most predominant spoligotype identified in prospectively recruited patients (Fig. 2). The Ethiopian L7 genotype (3/89, 3.4%) was identified only among stored isolates (Table 1).

In total 35 different spoligotype patterns were identified, and 107 (82.3%) grouped into 12 clusters (containing 2–42 isolates per cluster) with identical profiles. The largest clusters were SIT149 (T3-ETH, n = 42), SIT21 (CAS1-Killi, n = 19) and SIT25 (CAS1-Delhi, n = 17) (Supplementary Table S1).

3.2. Genotypic drug-susceptibility testing (DST) and mycobacterial spoligotypes

Of 130 MTBC isolates tested by MTBDR*plus*, RIF resistance was confirmed in only 89 (68.5%), with 87 (66.9%) found to be MDR-TB and two (1.5%) RIF mono-resistant. Three (2.3%) isolates were INH mono-resistant, and the remaining 38 did not show RMP- or INH-resistance-conferring mutations in the analyzed genes. From the 38 isolates that could not be confirmed as RR/MDR-TB by the LPA, 4 were from sputa testing false RR-TB by Xpert, and 34 were from the stored isolates previously identified as RR/MDR-TB by MTBDR*plus* assay. Regarding second-line drugs, only six RR/MDR-TB isolates (6.7%) were identified as resistant by MTBDR*sl* assay. Six isolates were fluoroquinolone-resistant (FQ-R) together with RR/MDR, of which three isolates

had additional resistance to second-line injectable drugs, such as kanamycin /amikacin/capreomycin-resistant isolates (i.e. extremely drug-resistant), including one isolate with low-level kanamycin resistance (data not shown).

T3-ETH, CAS1-Killi, CAS1-Delhi, and T- family were the most common RR/MDR-TB strains. The spoligotypes in the six FQ-R with or without additional SLI-R were SIT21, SIT149, and SIT50, representing the CAS1-Killi, T3-ETH, and Haarlem families, respectively.

3.3. Frequency of resistance-conferring gene mutations

Of 89 (68.5%) isolates harboring a mutation located in the *rpo*B gene, 57 (64.0%) had an S450L mutation (*rpoB*MUT3), 7 (7.9%) showed mutation H445Y (*rpoB*MUT2A), 3 (3.4%) showed D435V (*rpoB*MUT1), and 21 had undefined or rare mutations, indicated by the absence of 1 or more *rpo*BWT bands. One isolate (T3-ETH with SIT149 spoligotype) was suggestive of heteroresistance (i.e. presence of all *rpo*B wildtype bands and the *rpo*BMUT3 mutation band) (Table 2).

Among 90 INH-resistant isolates, 79 (87.8%) had the *kat*G S315T mutation (*kat*GMUT1 band), which represents a relatively high level of resistance. There were only three isolates that showed mutation (c-15t) at the *inh*A gene, often associated with low-level INH resistance. No double mutation with *kat*G and *inh*A genes was found (Table 2).

Of the six FQ-resistant isolates, four showed a *gyrA* D94G mutation (*gyrA*MUT3C band), one had an S91P mutation (*gyr*AMUT2 band), and one missed the *gyr*AWT3 and *gyr*BWT bands. Resistance to the SLIs was characterized by *rrs*MUT1 in one case and *eis*WT3 in the second (Table 2).

3.4. Geographical distribution of RR/MDR and FQ-(SLI)-R isolates among lineages

Our RR/MDR-TB isolates originated from 48 towns of the Amhara administrative region (Fig. 3). A high proportion of MDR-TB isolates was found in Gondar (n = 9, 6.9%), Alefa (n = 8, 6.2%), Dessie (n = 7, 5.4%), and Dembiya (n = 5, 3.8%). The three FQ-R isolates were found in North Gondar (Makesegnit; L3-CAS1-Killi), South Wollo (Kalu; L4-Haarlem), and West Gojam (Guzamen; L3-CAS1-Killi), while the other three FQ/SLI-R isolates were found in South Wollo (Dessie and Batti; L1-Family33) and East Gojam (Durbette; L4-T3-ETH) (Fig. 3).

3.5. Association of drug resistance and mycobacterial lineages

RR/MDR-TB was significatively associated with East African Indian L3 (odds ratio [OR] = 0.45; 95% CI, 0.21–0.98; $P = 0.044$) and Euro-American L4 (OR = 2.27; 95% CI, 1.08–4.79; $P = 0.031$). In addition, the analysis shows that RIF resistance was significantly associated with CAS1-Killi (OR = 12.7; 95% CI, 1.26–128.78; $P = 0.031$) and T3-ETH (OR = 14.2; 95% CI, 1.81–112.32; $P = 0.012$) strains, and similarly INH resistance was also associated with CAS1-Killi (OR = 27.0; 95% CI, 1.82–399.23; $P = 0.016$) and T3-ETH (OR = 19.5; 95% CI, 2.29–165.76; $P = 0.007$) strains (Table 3).

4. Discussion

In Ethiopia, the emergence of drug-resistant TB is the major barrier for TB prevention and control program. In this study, 89.2% of clinical RR/MDR-TB isolates were distributed in four main MTBC lineages, with Euro-American L4 (55.4%) and East African Indian L3 (40.0%) found to be the most prevalent. This result is similar to other studies [22,23] that reported a significant increase in the frequency of these lineages in drug-resistant TB in Ethiopia. In our findings, however, the high proportion of L4 and L3 amongst the RR/MDR-TB isolates might not be explained by these organisms being more prone to being drug-resistant but could rather be a consequence of their overall high prevalence in TB patients in Northwest Ethiopia [24], in which the predominantly circulating lineages might be influencing the emergence of drug resistance. MTBC strains circulating in TB endemic area implies not only a risk for enhancing ongoing transmission but also the capacity to spread resistant strains that can escape the effects of anti-TB drug regimens. Additionally, local factors including poor compliance with treatment and high rates of irregular medication have been known to facilitate the emergence of drug resistant MTBC strains [2].

Our findings also showed a significant association between the spoligotypes of the T3-ETH (SIT149) and CAS1-Killi (SIT21) with RR/MDR. Likewise, prior studies in Ethiopia showed that these spoligotypes were more often linked with drug resistance-conferring mutations and clonal expansion in Ethiopia [23]. Perhaps, the connection of certain MTBC genotypes with MDR could be attributed to their genetics and enhanced intrinsic ability to acquire resistance to anti-TB drugs [25] and establish in the native population. However, the correlation between the majority of MTBC genotypes and drug resistance, except for Beijing genotypes, is still undefined in TB endemic areas [26]. Possible risk factors, such as internal population migration, prolonged exposure to anti-TB drugs, and urbanization might also contribute to the increase of these MTBC spoligotypes and create favorable ecological conditions for MDR strain transmission, as evidenced by different reports [27]. This also suggests that resistant strains in regions with ineffective control strategies are linked with higher fitness and clonal expansion.

We did not identify drug resistance-conferring mutations among the Ethiopian restricted L7, which were susceptible to RIF, INH, FQ, and SLI drugs in this study, similar to earlier studies in Northwest Ethiopia [24,28], although the frequency of Ethiopian L7 was very low in our analysis. The reason for the absence of gene mutation is not clearly understood, but the effect of other mechanisms, such as efflux pumps, might be linked to these differences [29]. The detection of resistance in initial routine testing (the Xpert assay) may also be a difference in genomic variant of MTBC lineages [30], which might not be represented by a mutant probe on the LPA assay. However, the reason for the discordance results remains unclear. A study also found that a false susceptible LPA result was most probably due to the presence of heteroresistance with loss of minor variant-resistant subpopulations during subculture [31]. Future whole-genome sequencing studies need to be conducted to elucidate the detailed drug-susceptibility profiles of L7 and other lineages in the area.

Similar to previous studies in Ethiopia, the most common resistance-conferring mutations we observed were *rpoB* S450L for RIF and *katG* S315T for INH [32,33]. These mutations have been consistently associated with high-level resistance [11]. However, we also identified borderline *rpo*B mutations D435Y, H445R, and L452P. Different studies displayed that uncertain *rpo*B mutations from the RIF-resistant MTBC isolates are often frequent and have substantial effects on the prevalence and outcomes of RR/MDR-TB [34,35]. Short treatment regimens are used to reduce the emergence of antimicrobial resistance in *M. tuberculosis* [36]. Since 2018, in Ethiopia, patients with RR/MDR-TB have started using the short (9–11 months) MDR-TB treatment regimen unless they have resistance to core second-line anti-TB agents or meet other exclusion criteria [37].

While FQ and SLI resistance was relatively infrequent in this study, the mutations observed at codon 94 of the *gyrA* gene confer high-level resistance [38], conferring poor treatment outcomes in MDR-TB patients and increasing the emergence of (pre-)XDR-TB in the area. In fact, geographic variation in *gyrA* mutations have been well described in different regions [39]. There is increasing evi-

Table 2
Frequency of resistance-conferring mutations and their distribution in *Mycobacterium tuberculosis* complex (MTBC) families

Drug(s) resistance	Mutations occurred wildtype missed	mutant develop	mutation included in LPA[a]	Indo-Oceanic (L1) Family33(n)	Manu(n)	East African Indian (L3) CAS1-Delhi(n)	CAS1-Killi(n)	CAS(n)	Euro-American (L4) Haarlem(n)	T-family(n)	T3-ETH(n)	LAM(n)	Ethiopian lineage (L7) X-family(n)	ETH1-family(n)	Total number
RIF (n = 89)	*rpoB*WT8	-	L452P	2	-	-	-	1	-	-	1	-	-	-	4
	*rpoB*WT8	*rpoB*MUT3	S450L	-	1	5	11	1	4	7	25	1	2	-	57
	*rpoB*WT7	*rpoB*MUT2A	H445Y	-	-	1	-	-	-	1	5	-	-	-	7
	*rpoB*WT7	-	H445R	-	-	2	-	-	1	1	3	-	-	-	7
	*rpoB*WT3 & WT4	-	D435Y	-	-	-	6	-	-	-	-	-	-	-	6
	*rpoB*WT3 & WT4	*rpoB*MUT1	D435V	-	-	-	-	-	-	-	3	-	-	-	3
	*rpoB*WT2 & WT3	-	Q432P	-	-	2	-	-	-	-	-	-	-	-	2
	*rpoB*WT3	-	E429H	-	-	2	-	-	-	-	-	-	-	-	2
	-	*rpoB*MUT3	S450L	-	-	-	-	-	-	-	1	-	-	-	1
INH (n = 90)	*katG*WT	-	-	-	-	-	3	-	-	2	-	-	-	-	5
	*katG*WT	*katG*MUT1	S315T1	2	1	11	15	1	4	8	36	1	-	-	79
	*katG*WT	*katG*MUT2	S315T2	-	-	-	-	-	-	-	3	-	-	-	3
	*inhA*WT1	*inhA*MUT1	C−15T	-	-	1	-	-	-	-	-	-	2	-	3
FQ (n = 3)	*gyrA*WT3	*gyrA*MUT3C	D94G	-	-	-	1	-	1	-	-	-	-	-	2
	-	*gyrA*MUT2	S91P	-	-	-	1	-	-	-	-	-	-	-	1
FQ and SLI (n = 3)	*gyrA*WT3 *rrs*WT1	*gyrA*MUT3C *rrs*MUT1	D94G A1401G	2	-	-	-	-	-	-	-	-	-	-	2
	*gyrA*WT3 *gyrB*WT	-	-	-	-	-	-	-	-	-	1	-	-	-	1
	*rrs*WT1	-	C1402T												
	*eis*WT3	-	C−2A												

INH, isoniazid; RIF, rifampicin; FQ, fluoroquinolones; SLIDs, second-line injectable drugs (AMK, amikacin; CAP, capreomycin; KAN, kanamycin)
[a] Mutations were interpreted based on GLI interpretation as described in the guideline [19] and using consensus numbering system [22].

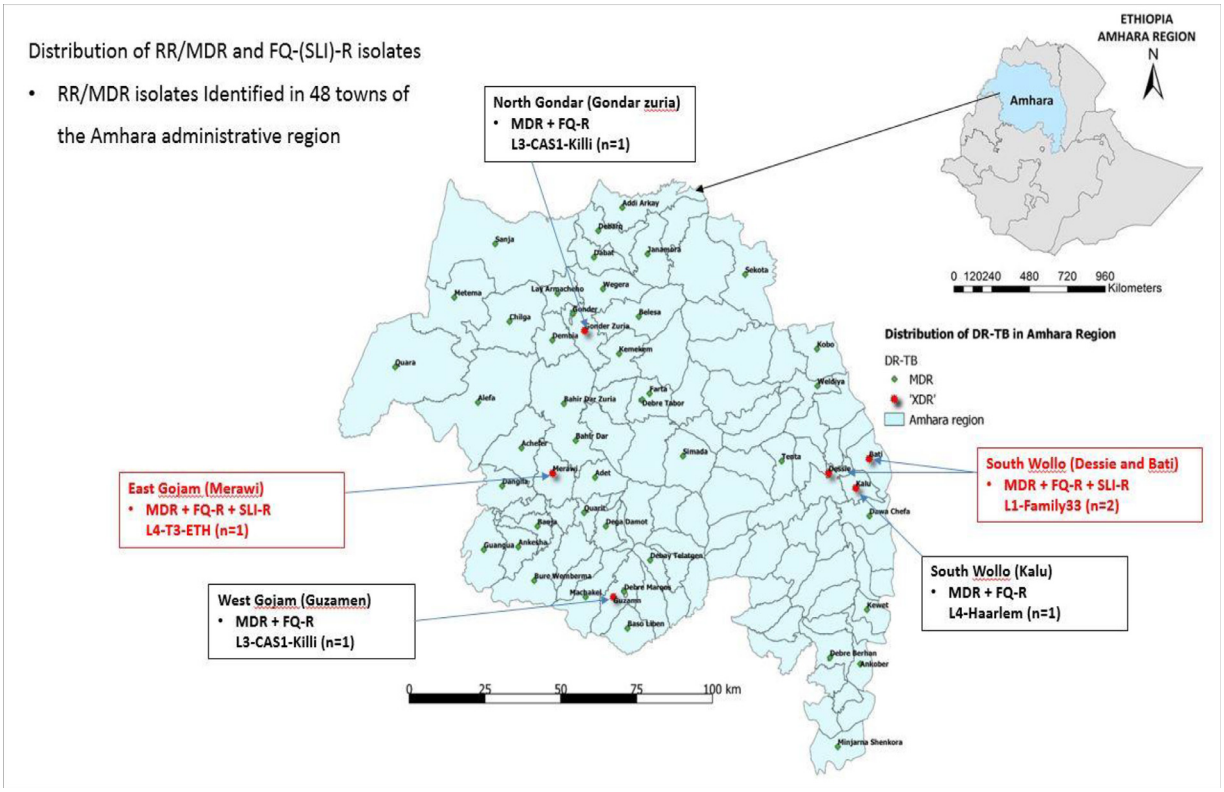

Fig. 3. Distribution of RR/MDR-TB isolates in the different districts of the study area, Northwest Ethiopia.
DR-TB, Drug-resistant tuberculosis; MDR, multidrug-resistant; RR, rifampicin-resistant; FQ-R, fluoroquinolone resistance; SLI-R, second-line injectables drug-resistance.

Table 3
Association of drug resistance and mycobacterial lineages and/or families

Lineages (L) and Family	RIF-resistant, N = 89 (%)	*P* value[a]	[95% CI] [a]	INH-resistant, N = 90 (%)	*P* value[a]	[95% CI] [a]
Indo-Oceanic (L1) (N = 3)	3 (3.4)			3 (3.3)		
Family33 (n = 2)	2 (2.2)	-	-	2 (2.2)	-	-
Manu (n = 1)	1 (1.1)	-	-	1 (1.1)	-	-
East African Indian (L3) (N = 52)	31 (34.8)			31 (34.4)		
CAS1-Delhi (n = 28)	12 (13.5)	0.905	[0.16–7.82]	12 (13.3)	0.905	[0.16–7.82]
CAS1-Killi (n = 19)	17 (19.1)	0.031	[1.26–128.78]	18 (20)	0.016	[1.82–399.23]
CAS (n = 5)	2 (2.2)	1.000	[0.08–12.55]	1 (1.1)	0.497	[0.02–6.34]
Euro-American (L4) (N = 72)	55 (61.8)			56 (62.2)		
Haarlem (n = 6)	5 (5.6)	0.158	[0.46–122.70]	4 (4.4)	0.383	[0.25–35.33]
T-family (n = 17)	9 (10.1)	0.613	[0.22–12.81]	10 (11.1)	0.463	[0.28–16.37]
T3-ETH (n = 42)	38 (42.7)	0.012	[1.81–112.32]	39 (43.3)	0.007	[2.29–165.76]
LAM (n = 2)	1 (1.1)	0.810	[0.05–40.63]	1 (1.1)	0.810	[0.05–40.63]
X-family (n = 5)	2 (2.2)	-	-	2 (2.2)	-	-
Ethiopian lineage (L7) (N = 3)	0 (0.0)			0 (0.0)		
ETH1 family (n = 3)	0 (0.0)	-	-	0 (0.0)	-	-

CAS, Central Asian; CI, Confidence Interval; L, lineage; LAM family, Latin American-Mediterranean.
[a] *P* value and 95% CI were calculated using the two-sample proportion test.

dence that the occurrence of FQ resistance in Ethiopia within MDR-TB isolates can be attributed to its common use in the treatment of respiratory tract infections and other bacterial diseases [40]. This might also increase the risk of FQ (hetero)-resistance development, which is difficult to detect and negatively affects the efficiency of MDR-treatment.

Our study had some limitations. The study included only MTBC isolates from patients referred to the TB culture laboratories, which might not be representative of MDR-TB strains circulating in Northwest Ethiopia. Because of the lack of resources, we did not conduct the phenotypic drug-susceptibility testing for all resistant MTBC strains to compare with the molecular resistant results in this study. We used only spoligotyping for the characterization of the MTBC strains, which might have resulted in lower resolution of the genotypes. In addition, we did not genotype all retrospectively retrieved isolates nor could we test the isolates for their genotypic susceptibility because of restricted permission to use only the available re-grown isolates. Discordant results from initial testing were a major study limitation.

5. Conclusion

Our findings displayed high genetic diversity of MTBC among the RR/MDR isolates analyzed, with a predominance of L4-T3-ETH, L3-CAS1-Delhi, and L3-CAS1-Killi families. The most common drug resistance-conferring mutations were occurred on codons of *rpoB* (S450L) and *katG* (S315T). The study also showed low proportion of mutations on the inhA gene promotor and the *gyrA* (D94G) and *rrs* (A1401G) in L4 and L3 MDR isolates. L7 was less common in drug-resistant isolates. Therefore, our findings highlight

that L7 and other lineages with few borderline mutations need further investigation to understand their effects on the treatment of RR/MDR-TB patients in the area.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Ethical approval

Ethics approval for this study was granted by the University of Gondar in Ethiopia, the Institute of Tropical Medicine (Antwerp), and the University of Antwerp in Belgium. Permission for use of routinely collected samples and data was obtained from the University of Gondar comprehensive specialized hospital and Amhara Public Health Institute TB laboratory to retrieve patient data and isolates from storage. Confidentiality was maintained throughout the conduct of the study by use of codes in data management and analysis. The final research data was accessible only for the researchers.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.11.012.

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