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Mycobacterium tuberculosis in central Ethiopia: drug sensitivity patterns and association with genotype

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Abstract

Drug resistance tuberculosis (TB) and the emergence of multidrug resistant (MDR) isolates are significant concerns regarding TB control programs in several countries. This study was undertaken to evaluate the drug sensitivity of *Mycobacterium tuberculosis* and to assess its association with strains and lineages of *M. tuberculosis.*

A total of 279 *M. tuberculosis* strains isolated from Central Ethiopia were tested for their drug sensitivity patterns to first line TB drugs using the conventional proportion method on Löwenstein Jensen media. The association between drug sensitivity and strain type was assessed on 263 isolates of the 279 isolates.

Of the 268 *M. tuberculosis* isolates obtained from new cases, 209 (78%) were susceptible to first line TB drugs, and 59 (22.2%) bacterial isolates were resistant to at least one of the first line drugs. The highest mono-resistance (7.5%) pertained to streptomycin (STM). Remarkably, seven of eleven isolates (63.6%) previous treatment for TB were resistant to at least one of the first line drugs. The prevalence of MDR-TB was 1.5% (4/268) for newly identified TB cases, all of which were members of the Euro-American Lineage. There was no statistically significant association ($P > 0.05$) between drug sensitivity, and either strains, sub-lineages or main lineages of *M. tuberculosis*.

A significant proportion of *M. tuberculosis* was resistant to at least one first line anti-TB drug. Moreover, the frequencies of resistance to either isoniazid or rifampicin were high compared to data that were previously reported in some part of the country.

Keywords: Drug resistance, genotype of *M. tuberculosis*, lineages, multi-drug resistance, proportional method
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Introduction

Tuberculosis (TB) causes illness in millions of people in each year and is ranked second to HIV/AIDS as a leading cause of death from an infectious disease worldwide [1]. The emergence of drug-resistant TB poses a challenge to healthcare systems, especially in low-income countries, and has exacerbated the situation as it pertains to the effective treatment of the disease worldwide. Inappropriate drug regimens, patient defaulting, previous anti-TB treatment, delays in diagnosis, treatment of the disease and primary infections with drug-resistant or multidrug-resistant (MDR) TB strains and poor infection control practices have been identified as major contributing factors for the spread of drug-resistant TB [2].

Worldwide, an estimated 480 000 people developed MDR-TB in 2013. MDR-TB is defined as resistant to two first-line anti-TB drugs, isoniazid (INH) and rifampicin (RIF). Globally, an estimated 3.5% of new cases and 20.0% of previously treated cases have MDR-TB [3]. India, China and the Russian Federation account for more than 54% of the MDR-TB cases [3]. On average, an estimated 9.7% of patients with MDR-TB have extensively drug-resistant (XDR) TB [3]. XDR-TB is resistant to any fluoroquinolones and at least one of three injectable

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drugs, capreomycin, kanamycin, and amikacin, in addition to INH and RIF [4]. XDR-TB emerges when second-line drugs are misused to treat MDR-TB. Nearly half a million cases of MDR-TB emerge every year worldwide, of which ~50 000 are XDR-TB [4]. According to the World Health Organization (WHO), XDR-TB had been reported in 105 countries by the end of 2012 [3]. Fourteen of these countries reported more than 10% of XDR-TB cases. Among these countries, the proportion of MDR-TB cases with XDR-TB was highest in Belarus (29% in 2014), Georgia (15% in 2014), Latvia (19% in 2014) and Lithuania (25% in 2013) [3].

Ethiopia is one of the 30 high TB and MDR-TB burden countries in the world [3]. According to the WHO report, the prevalence and incidence of all forms of TB are 200 and 207 per 100 000 population respectively; the mortality due to TB was estimated to be 33 per 100 000 of the population [3]. The same report showed that 1.6% of newly diagnosed TB patients and 12% of previously treated patients had MDR-TB. These data warrant the identification of drug-resistant strains and monitoring their transmission in the community to contain their spread. Specifically, it is of interest to know whether specific genotypes of *Mycobacterium tuberculosis* (strains or lineages) are responsible for the development of the majority of drug resistances in a distinct geographical region. Therefore, the objective of the present study was to evaluate drug sensitivity patterns of *M. tuberculosis* strains isolated from central Ethiopia and to assess the association of drug resistance with different strains of *M. tuberculosis*.

Methods

Source of isolates

A total of 279 *M. tuberculosis* strains isolated from smear-positive TB patients in central Ethiopia were included in this study. Sample collection was performed at three hospitals located in central Ethiopian sites.

Study design

This study was a health institution–based cross-sectional study that was conducted on newly and previously treated adults (>18 years old) with TB between October 2012 and September 2013. The sample size was calculated on the basis of the sampling method recommended by WHO guidelines for surveillance of drug resistance TB [5].

Culture

Culture was done according to WHO guidelines [6]. Briefly, morning and spot sputum samples were collected before the start of treatment regimens. Sputum samples were pooled, homogenized and decontaminated with equal volumes of 4% NaOH. An aliquot of 100 µL of the suspension was inoculated onto sterile Löwenstein-Jensen medium. The inoculated media were then incubated at 37°C in a slanted position for 1 week and an upright position for 4 to 5 weeks. The bacteria growth was read every week until the eighth week of culture.

Preparation of DNA for molecular typing

Colonies were removed from the surface of Löwenstein-Jensen medium and suspended in 200 µL of sterile double-distilled water. Thereafter, the colonies and water were mixed thoroughly; then the mixture was heated to 80°C for 1 hour in a water bath. This is followed by centrifugation, after which the supernatant was collected and used for amplification.

Identification

Identification of *M. tuberculosis* from the other members of the *M. tuberculosis* complex species was done using region of difference (RD)-9 PCR [7], which was performed on heat-killed bacterial cells using three primers: RD9 flank F, RD9 Internal R and RD9 flank R [8]. PCR amplification was performed as indicated by Berg *et al.* [8] by commercially available kit (Qiagen) using three primers: RD9flankF, RD9IntR and RD9flankR. The presence of RD9 (i.e. *M. tuberculosis*) gives a product size of 396 bp (RD9 FlankF + RD9 Internal), and its absence (*Mycobacterium africanum, M. bovis*) gives a product size of 575 bp (RD9 FlankF + RD9 FlankR).

Conventional drug susceptibility testing (DST)

DST was performed for four first-line drugs—INH, streptomycin (STM), RIF, and ethambutol (EMB)—using the indirect proportion method on Löwenstein-Jensen medium. The critical concentrations for each drug were 0.2, 4, 40 and 2µg/mL for

TABLE 1. Demographic characteristics of 279 study subjects and association with drug sensitivity patterns of tuberculosis strains

Characteristic	Any drug resistance: Yes, *n* (%)	Any drug resistance: No, *n* (%)	Total, *n* (%)	COR (95% CI)	p
Sex					
Male	34 (51.5)	121 (56.8)	155 (55.6)	1	
Female	32 (48.5)	92 (43.2)	124 (44.4)	1.238 (0.712–2.153)	0.450
Age group					
18–28 years	37 (56)	68 (31.9)	105 (37.6)	0.350 (0.168- 0.761)	0.005
29–39 years	12 (18.2)	63 (29.6)	75 (26.9)	0.353 (0.161–0.776)	0.010
40–50 years	10 (15.2)	52 (24.4)	62 (22.2)	0.429 (0.172–1.071)	0.070
>50 years	7 (10.6)	30 (14.1)	37 (13.3)	1	
Treatment history					
New patient	59 (89.4)	209 (98.1)	268 (96.1)	1	
Previously treated	7 (10.6)	4 (1.9)	11 (3.9)	0.161 (0.0460–0.570)	0.005
Study area					
Woliso	31 (47)	100 (47)	131 (47)	1	
Atat	8 (12.1)	39 (18.3)	47 (16.8)	0.662 (0.280–1.565)	0.348
Fiche	27 (40.9)	74 (34.7)	101 (36.2)	1.177 (0.648–2.138)	0.593

CI, confidence interval; COR, crude odds ratio.

TABLE 2. Drug sensitivity patterns of *Mycobacterium tuberculosis* isolated from central Ethiopia to first-line antituberculosis drugs using conventional method ($n = 279$)

Drug resistance pattern	New cases ($n = 268$), *n* (%)	Treated cases ($n = 11$), *n* (%)
All tested	268 (100)	11 (100)
Susceptible	209 (78.0)	4 (36.4)
Any resistance	59 (22.0)	7 (63.6)
Monoresistance	46 (17.2)	4 (36.4)
MDR	4 (1.5)	0 (0.0)
RIF only	5 (1.9)	1 (9.1)
INH only	17 (6.3)	0 (0.0)
EMB only	4 (1.5)	1 (9.1)
STM only	20 (7.5)	2 (18.2)
RIF + INH	2 (0.7)	0 (0.0)
INH + STM	4 (1.5)	2 (18.2)
INH + EMB	0 (0.0)	1 (9.1)
EMB + STM	3 (1.1)	0 (0.0)
INH + EMB + STM	1 (0.4)	0 (0.0)
INH + RIF + STM	2 (0.7)	0 (0.0)
Any RIF	9 (3.4)	1 (9.1))
Any INH	26 (9.7)	3 (27.3)
Any EMB	8 (3.0)	2 (18.2)
Any STM	30 (11.2)	4 (36.4)

EMB, ethambutol; INH, isoniazid; MDR, multidrug resistant; RIF, rifampicin; STM, streptomycin.

INH, STM, RIF and EMB respectively. The experiments were performed following standard protocols [9].

The interpretation of the results was done by comparing amount of growth on control media and drug-containing media. A strain was considered resistant when bacterial growth on a drug containing media $\geq$1% [9].

Statistical analysis

Statistical analysis was performed by Stata 12 software (StataCorp, College Station, TX, USA). Descriptive analysis, frequencies and odds ratios (OR) with 95% confidence intervals (CIs) were calculated. In order to determine independent risk factors, ORs and 95% CIs were calculated using logistic regression analysis. In the logistic regression model demographic variables, treatment history and drug resistance were include as confounding variables. Results with p values of <0.05 were considered statistically significant.

Results

Sociodemographic characteristics

The sociodemographic characteristics of the study participants are summarized in Table 1. Of the 279 total isolates included in the study, 56.6% (n = 155) were isolated from men, and 44.4% (n = 124) were isolated from women. The majority of the study participants (96.1%) were new patients. The mean age of the patients was 34.5 years (Table 1).

Identification

The isolates were analysed by RD9 PCR, and the results indicated that all of the isolates had intact RD9, thus implying that the isolates were *M. tuberculosis*.

Drug resistance patterns to first-line anti-TB drugs by conventional method

The results of drug sensitivity tests for *M. tuberculosis* strains isolated from patients in central Ethiopia are presented in Table 2. Drug sensitivity tests to four first-line anti-TB drugs were performed for a total of 279 *M. tuberculosis* isolates. Of the 268 *M. tuberculosis* isolates isolated from newly diagnosed cases, 78.0% (209/268) were susceptible to the four drugs, but 22.0% (59/268) of them were resistant to one or more drugs. Of the 11 previously treated cases, 36.4% (4/11) were susceptible to all four drugs, while 63.6% (7/11) were resistant to at least one drug. MDR was observed in 1.5% (4/268) of the new isolates. Monoresistance was observed for 17.2% (46/268) of the new cases and 36.4% (4/11) of the cases previously treated for a TB infection. The highest proportion of monoresistance in new cases was observed to STM (7.5%,), followed by INH (6.3%), RIF (1.9%) and EMB (1.5%). The percentages for any resistance to RIF, INH, EMB and STM, including cases with resistance to more than one of the four drugs, were 3.5% (10/279), 10.4% (29/279), 3.5% (10/279) and 12.2% (34/279) respectively. Not a single strain was resistant to all four drugs tested (Table 2).

TABLE 3. Drug resistance stratified by *Mycobacterium tuberculosis* lineage

Lineage (*n*)	RIF only	INH only	EMB only	SMonly	RIF + INH	INH + EMB	INH + SM	EMB + SM	INH + EMB + SM	INH + RIF + SM	Total resistance, *n* (%)
CAS1-Delhi (14)		2			0	0					2 (14.3)
CAS1-Kili (2)		1			0	0					1 (50)
EAI- (2)		0		1	0	0					1 (50)
H (26)	2	0	1	4	0	0		1			8 (30.8)
LAM5 (3)		0		1	0	0					1 (33.3)
Manu (71)	1	1	1	5	0	0	4	1		2	15 (21.1)
T (131)	2	12	2	9	2	1	2	1	1		32 (24.4)
Turkey (6)		0		0	0	0					0
X (8)		0	1	0	0	0					1 (12.5)
Total	5	16	5	20	2	1	6	3	1	2	61

EMB, ethambutol; INH, isoniazid; RIF rifampin; SM, streptomycin.

TABLE 4. Association between drug resistance and genotype of *Mycobacterium tuberculosis* isolates from central Ethiopian tuberculosis patients ($n = 263$)

		Any drug resistance				
Characteristic	**Variable**	**Sensitive**	**Resistant**	**Total**	**COR (95% CI)**	**p**
Major lineage by CBN	EA	181	52	233	1	
	EAI	13	3	16	0.803 (0.221–2.926)	0.740
	IO	7	5	12	2.486 (0.758–8.159)	0.133
	MA	1	1	2	3.481 (0.214–56.609)	0.381
Sublineage/clade	CAS1-Delhi	12	2	14	1	
	CAS1-Kili	1	1	2	6.000 (0.257–140.045)	0.265
	EAI	1	1	2	6.000 (0.257–140.045)	0.265
	H	18	8	26	2.667 (0.481–14.789)	0.262
	LAM	2	1	3	3 .000 (0.177–50.784)	0.447
	Manu	56	15	71	1.607 (0.324–7.974)	0.562
	T	99	32	131	1.939 (.4.12–9.129)	0.404
	Turkey	6	0	6		
	X	7	1	8	0.857 (0.065–11.256)	0.907
Dominant strain	Orphan	41	12	53	1	
	SIT 53	34	9	43	0.904 (0.341–2.401)	0.840
	SIT 149	25	12	37	1.640 (0.639–4.204)	0.303
	SIT 54	27	4	31	0.506 (0.148–1.734)	0.279
Clustering	No	39	18	57	1	
	Yes	163	43	206	0.572 (0.298–1.097)	0.93

CBN, conformal Bayesian network; CI, confidence interval; COR, crude odds ratio; EA, Euro-American; EAI, East African–Indian; IO, Indo-Oceanic; MA, *Mycobacterium africanum*.

Association of drug resistance and demographic characteristics of study subjects

The result of the analysis of the association of the drug sensitivity patterns and sociodemographic characteristics of the subjects used as sources of the isolates is presented in Table 1. Anti-TB drug resistance was observed in male and female patients, with 51.5% (34/279) and 48.5% (32/279) respectively, and the difference in drug resistance between the two was statistically insignificant (p 0.450). High frequency of resistance was observed in the age group 18 to 28—significantly higher compared to other age groups (p 0.005) (Table 1).

Relationships of drug-resistant phenotypes with *M. tuberculosis* genotypes

The relationship between the drug-resistant phenotypes and the *M. tuberculosis* lineages and strains was analysed for 263 isolates (Table 3). The majority of the isolates were members of sublineages T, 49.8% (131/263), and Manu, 27.0% (71/263). The anti-TB drug resistances of the isolates in these two sublineages were 24.4% and 21.1% respectively (Table 3). The majority (12/16) of the INH monoresistant isolates and the majority (9/20) of the STM monoresistant isolates were members of the T sublineage.

The association between drug sensitivity patterns of *M. tuberculosis* isolates and the main lineages of *M. tuberculosis* is presented in Table 4. The frequencies of drug resistances were 19.8% (52/263), 1.1% (3/263), 1.9% (5/263) and 0.4% (1/263) in Euro-American, Indo-Oceanic, East African–Indian and *M. africanum* respectively. Although the highest frequency of resistance was observed for the T sublineage, it was not statistically significant (p 0.404) compared to other sublineages. There was no significant association (p >0.05) between drug resistance and either main lineage, sublineage or dominant strain (Table 4).

Discussion

The present study was conducted to evaluate the drug sensitivity patterns of *M. tuberculosis* isolated from TB patients visiting health institutions in three towns in central Ethiopia. A total of 279 *M. tuberculosis* isolates were included in this study. In addition, the association between drug sensitivity patterns and the genotype of *M. tuberculosis* was examined.

The result of this study revealed that 22% of the isolates from newly diagnosed TB cases and 63.6% of isolates from previously treated TB cases were resistant to at least one of the four anti-TB drugs investigated here. Comparable frequencies of resistance for newly diagnosed TB cases were reported from other areas in Ethiopia, such as Addis Ababa [10] and eastern Ethiopia [11]; higher frequencies of resistance were reported from other regions [12–14]. Lower frequencies of drug resistances were reported from yet other Ethiopian regions [15–17]. Two studies from other East African regions, including Uganda [18] and the city of Nairobi [19], reported higher frequencies of resistance, 28.6 and 30% respectively. The differences in overall prevalence of drug resistance among the different study settings could be due to difference in sample size, design of the study, study participant, access to healthcare facilities and effectiveness of TB control programs.

In the present study, 17.2% of the isolates from newly diagnosed cases and 36.4% from the retreated cases were

monoresistant to any one of the four first-line drugs, most commonly to STM (7.5%) and INH (6.3%). On the other hand, a higher frequency (27.7%) of monoresistance to any one of the four first-line drugs was reported in another study conducted in the country [20]. INH monoresistance (6.3%) recorded by the present study was comparable to that reported by studies conducted in other African countries such as the Central African Republic [21] and Somalia [22], which reported 5.8 and 5.7% respectively. In an eastern Ethiopian study [11], 9.5% monoresistance to INH was recorded. The INH monoresistance can increase the possibility of MDR-TB if RIF resistance also rises. Monoresistance to INH should be monitored in order to minimize the spread of MDR-TB strains.

In this study, the frequency of monoresistance to RIF was 1.9% for newly diagnosed cases. This result is comparable to those of other studies conducted in eastern Ethiopia [11] and Addis Ababa [10]. Even though RIF monoresistance is relatively low according to the present study, monitoring the appropriate use of this drug is also important to avoid the development of MDR-TB. Monoresistance to EMB (1.5% of the cases) was higher compared to studies in eastern Ethiopia [11], Addis Ababa [14], Burkina Faso [23] and Uganda [24]. In contrast, monoresistance to EMB was higher in another study for Addis Ababa (3.5%) [10].

The prevalence of MDR-TB in this study was 1.5%, which is in agreement with reports of other surveys conducted in Ethiopia [15,16]. Other studies reported a higher frequency of MDR-TB [14,20]. Higher MDR cases observed in both studies may be due to small sample size and difference in study participant because samples that were taken from TB specialized hospital [14] might have higher MDR-TB than the finding of this current study. Control of MDR-TB requires an effective TB control program, including a regular supply of anti-TB drugs, well-organized patient diagnosis, appropriate treatment, follow-up and good patient adherence. Higher frequency of drug resistance reported in the age group 18 to 28 years is in agreement with a WHO report [4]. According to this WHO report, two thirds of TB cases are estimated to occur among young people. In Ethiopia, there are no regional bacterial culture and DST facilities for routine diagnosis of drug resistance. Consequently, drug-resistant TB is often diagnosed only after prolonged treatment with first-line anti-TB drugs and clinical recognition that treatment has failed. Therefore, identification of anti-TB drug–resistant strains and understanding the patterns of transmission of such strains in the community are important to control epidemiologic outbreaks and further aggravation of MDR and XDR drug resistance.

Many studies [25–28] showed that drug-resistant phenotypes are not equally distributed among *M. tuberculosis* genotypes. In this study, resistance to first-line anti-TB drugs was highest in Euro-American lineage and in the T sublineage, but the difference was not statistically significantly higher than the frequencies of resistance in other lineages and sublineages. Thus, in the present study, no association was observed between *M. tuberculosis* genotypes and their resistance to the first-line anti-TB drugs. Other studies performed in the northern part of the country showed that Haarlem strains were more likely to be resistant to any of the four first-line anti-TB drugs compared to other lineages [27].

Although our study has established the first data on drug resistance of *M. tuberculosis* circulating in specific sites of study area (southwestern part of central Ethiopia), it has some limitations. One of the limitations was selection; the study did not include all public health facilitates (in addition to hospitals) as a result of resource restrictions. In addition, the study did not include data from the possible association between HIV infection and anti-TB drug resistance.

In conclusion, a significant proportion of *M. tuberculosis* was resistant to at least one or more first line anti-TB drug. Moreover, resistance to either INH or RIF was high compared to frequencies of resistance reported earlier in other parts of the country. On the other hand, no association was observed between the genotype of *M. tuberculosis* isolates and their drug sensitivity patterns.

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Conflict of Interest

None declared.

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