Original Article

Epidemiology of first-and second-line anti-tuberculosis drug resistance in new pulmonary tuberculosis cases in Addis Ababa metropolitan area, Ethiopia

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Abstract

Background: Conventional wisdom holds that the microbiological detection of *Mycobacterium tuberculosis* complex in clinical specimens via culture and phenotypic drug susceptibility testing allows people to be correctly diagnosed and ensures that an effective treatment regimen is selected.

Aim: The objective of this study was to provide a description of the drug resistance profiles of first-line and second-line anti-tuberculous drugs among individuals newly diagnosed with pulmonary tuberculosis in the Addis Ababa metropolitan area, located in Ethiopia.

Methods: A cross-sectional study was conducted between October 2019 and June 2021 on clinically suspected and bacteriologically confirmed pulmonary tuberculosis cases. The GeneXpert MTB/RIF assay was used to detect *Mycobacterium tuberculosis* complex and resistant to rifampicin. Mycobacterial culture and drug susceptibility testing were performed against 15 anti-tuberculosis drugs using the BD BACTEC™ MGIT™ 960 automated liquid culture system.

Results: A total of 156 *Mycobacterium tuberculosis* complex isolates were successfully recovered. Males account for 53.8 % [84/156]. Overall, 14.1% [22/156] of isolates were resistant to at least one drug, 85.9 % [134/156] of isolates were pan-susceptible, 7.1% [11/156] of isolates were mono-resistant strains, 5.8% [9/156] of isolates were multidrug-resistant strains, and 3.8% [6/156] of isolates were resistant to all first-line drugs. Interestingly, all isolates were susceptible to all recently recommended second-line drugs tested. Nevertheless, compared to the reference method, the rate of rifampicin resistance detected using GeneXpert MTB/RIF assay had a sensitivity, specificity, and accuracy of 100% [95% CI: 66.4 – 100], 94.6 [95% CI: 89.6 – 97.6], and 94.9 [95% CI: 90.2 – 97.8] respectively, with moderate level of agreement at Cohen kappa value of 0.667.

Conclusion: The rate of MDR-TB in new pulmonary TB cases remained high, at fivefold the national and nearly twofold the global estimated rate in this study. The rate of monoresistance was also high and alarming. The rate of resistance against second-line anti-TB drugs was absent and quite encouraging. The use of GeneXpert MTB/RIF assay alone for the detection of *M. tuberculosis* introduces 15.7% [34/216] false positivity and 47.1% [8/17] false rifampicin resistance. Treatment initiation with second-line medications without initial drug susceptibility test results may lead to erroneous decisions. [*Ethiop. J. Health Dev.* 2023; 37(2) 000-000]

Keywords: *Mycobacterium tuberculosis*, tuberculosis, anti-tuberculosis drugs, drug resistance.

Introduction

Tuberculosis (TB), caused by the *Mycobacterium Tuberculosis* (*M. tuberculosis*) complex, is the oldest disease known to affect humans with myriad clinical manifestations of diseases worldwide (1,2). *Mycobacterium. Tuberculosis* belongs to closely related mycobacterial species, including *M. bovis, M. africanum, M. microti, M. caprae, M. pinnipedii, M. canetti,* and *M. mungi* forming *M. tuberculosis* complex (3). It was widely believed that the genus *Mycobacterium* originated in animals and spread to humans during the Neolithic transition 150 million years ago (1, 4). However, recent evolutionary data suggested that *M. tuberculosis* emerged as a human pathogen in Africa and survived with modern human migrations approximately 70 000 years ago (2, 5). However, it was in 1882 when Robert Koch was able to isolate tubercle bacillus, that determined a milestone in the fight against TB (6).

According to the WHO Global Tuberculosis Report of 2022, an estimated 10.6 million persons became ill from TB worldwide, of which 5.3 million people were diagnosed with pulmonary TB, an estimated 1.6 million recorded deaths, and an estimated 4 million people with TB missed undiagnosed in 2021(7). The emergence of drug-resistant *M. tuberculosis* strains continues to be a clear threat to the realization of TB elimination (8). Globally, 3.6% of new TB cases and 18% of previously treated cases had multi-drug resistant/ rifampicin-resistant TB (MDR/RR-TB). Conversely, about 2 billion people are estimated to generate a latent TB infection (2,7,9). Ethiopia remained among the 30 TB and TB/HIV high-burden countries while transitioning out of the list of

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MDR/RR-TB for 2021 – 2025. The country recognizes an annual TB incidence rate of 143 per 100,000 and an estimated proportion of 1.1% and 12% MDR/RR-TB in new and previously treated cases, respectively (7).

Despite the remarkable campaign to control tuberculosis since 1948, the WHO declared TB a global emergency in 1993 and introduced DOTs as a control strategy in 1995 with the ambition to save lives and eliminate TB (6,10). Ethiopia adopted the directly observed therapy (DOT) strategy in 1997 and endorsed the 'Stop TB' and 'End TB' aspirational strategies of MDGs and SDGs (11). Albeit these concerted efforts, the rate of decline in TB incidence and mortality is lower than what would be required to reach the local as well as the global target of TB pre-elimination by 2035 and elimination by 2050.

The microbiological detection of TB is critical as it allows people to be correctly diagnosed, is necessary for drug resistance detection, and ensures that the most effective treatment regimen is selected as early as possible. Tuberculosis is mostly diagnosed using smear microscopy, rapid molecular diagnostics, culture, and phenotypic and genotypic drug susceptibility testing (DST) methods. However, the reference method for bacteriological confirmation and resistance detection is culture using commercially available liquid media (12).

The standard treatment regimens for susceptible TB strains in Ethiopia include Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol (HRZE) for 2 months intensive phase followed by Rifampicin and Isoniazid (RH) for 4 months continuation phases. While the standard shorter MDR-TB regimen updated in 2021 includes Bedaquiline, Levofloxacin, Clofazimine, Pyrazinamide, Ethambutol, High-dose isoniazid, and Ethionamide (BDQ -LFX-CFZ-Z-E-HH-ETO) for 4 – 6 months followed by Levofloxacin, Clofazimine, Pyrazinamide, Ethambutol (LFX-CFZ-Z-E) for 5 months continuation phase. The standard longer MDR-TB regimen includes Bedaquiline (BDQ) for 6 months, followed by Levofloxacin/Moxifloxacin, Linezolid, Clofazimine, and Cycloserine (LFX/MFX-LZD-CFZ-CS) for 18 months (7,9,11). On top of this, efficient treatment response surveillance is critical to measure treatment outcomes, effectively limit the transmission of resistant strains, and recognize drug resistance patterns. However, monitoring responses to anti-TB drugs using molecular assay is unsuitable, and culture and phenotypic DST are not routinely utilized in Ethiopia. This study aimed to describe the epidemiology of first-and second-line anti-TB drug resistance profiles among new pulmonary TB cases in the Addis Ababa metropolitan area, Ethiopia.

Materials and Methods

Study design, setting, and participants

From October 2019 to June 2021, a cross-sectional study was carried out at Saint Peter TB Specialized Hospital in Addis Ababa metropolitan city, as well as Sululta and Sendafa Health Centers in nearby towns. The study included newly diagnosed cases of pulmonary TB who met the clinical eligibility criteria. To confirm the presence of tubercle bacilli, all cases underwent bacteriological testing using the GeneXpert MTB/RIF assay.

Mycobacterial culture and phenotypic drug susceptibility testing were performed using BD BACTEC™ MGIT™ 960 automated liquid culture system: Becton Dickinson, Sparks, MD, USA, at the Ethiopia Public Health Institute National TB Reference Laboratory, Addis Ababa, Ethiopia. Sociodemographic and clinical data were captured using a structured and validated tool prepared for this study.

Inclusion and Exclusion Criteria

The inclusion criteria were new clinically suspected pulmonary TB cases whose suspicions were bacteriologically confirmed positive for TB infection. The exclusion criteria were being on treatment for TB within the last three months before the commencement of this study, treated for TB or have taken anti-TB medicines for more than one-month, extra-pulmonary TB cases who were unable to provide Sputum, and those who refused to participate were avoided from enrollment. Generally, the WHO case definition was applied, in which a new presumptive pulmonary TB case was defined as a newly identified episode of TB case who has never been treated for TB or has taken anti-TB medicines for less than a month (9, 11).

Sample Collection, Transport, and Storage

All clinically eligible newly recruited pulmonary TB cases who voluntarily demonstrated written informed consent were interrogated to provide two sputum samples of 3-5ml each in a sterile falcon tube of 50 ml capacity in front of a laboratory technologist. The first Sputum was collected upon enrollment and was analyzed using GeneXpert MTB/RIF assay immediately. The second Sputum was collected in the morning the following day. Both sputa were expectorated and without mechanical maneuver. Each patient's second sputum specimen was stored at 2 - 8 ^{0}C at St. Peter TB Specialized Hospital for a maximum of 24 hours after collection. Using appropriate packaging and reverse cold chain, all Sputum was transported to the National Tuberculosis Reference Laboratory for isolation of tubercle bacilli and drug susceptibility testing.

GeneXpert MTB/RIF assay

The GeneXpert MTB/RIF assay was performed according to the manufacturer's instruction, Cepheid, Sunnyvale, CA, USA (13). It is an automated in vitro diagnostic test using nested real-time PCR for the qualitative and semiquantitative detection of *M. tuberculosis* complex and simultaneous RR-TB detection.

Digestion, decontamination, and concentration of Sputum

Sputum specimens were digested and decontaminated using the standard NALC-NaOH method recommended by WHO and endorsed by the National TB Reference Laboratory, Tuberculosis Research Unit (12). Sodium citrate and phosphate buffer were used to maintain the stability of NALC and neutralize the NaOH homogenate, respectively. The supernatant was discarded, and the pellet was resuspended with sterile

phosphate-buffered saline. The mixture was used to inoculation of the BD BACTEC™ MGIT™ 960 tubes.

Before inoculation the specimens, 0.5ml of OADC: Oleic acid, albumin, dextrose, and catalase growth enrichment and 0.1ml of PANTA: polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin antibiotic mixture was added to the Mycobacteria Growth Indicator Tube that contains 7mL of modified Middlebrook 7H9 Broth base. Subsequently, the MGIT tubes were inoculated with 0.5 ml of the concentrated specimen suspension, tightly recapped, mixed well, and incubated at 37 °C in the BD BACTEC™ MGIT™ 960 instrument system. The Tubes were automatically monitored each for fluorescence development every hour for 42 days or until a positive signal was detected.

Isolation and identification of mycobacteria species

Sputum specimens were processed and cultured according to the national TB reference laboratory and WHO-recommended protocol using BD BACTEC™ MGIT™ 960 automated liquid culture system (12). For contamination detection, a brain heart infusion agar plate was used, and AFB smears were performed on all positive growths from each culture media to confirm the presence of tubercle bacilli under microscopic examination. To discriminate *Mycobacterium tuberculosis* complex from Mycobacteria other than tubercle bacilli, isolates were identified by TB Antigen MPT64 Rapid Test, SD Bioline, Republic of Korea. All mycobacteria isolates were stored at -20^{0}C until DST was performed.

First- and second-line drug susceptibility testing

Phenotypic DST for first-line anti-TB medicines was performed using the BD BACTEC™ MGIT™ 960 SIRE Kits for antimycobacterial susceptibility testing of *Mycobacterium tuberculosis* from culture. The critical concentrations of drugs used were: 2.0µg/ml for STM; 0.1µg/ml for INH; 1.0µg/ml for RIF and 5.0µg/ml for EMB as recommended by WHO (14). PZA was not included in the DST.

Phenotypic DST for second-line anti-TB drugs include fluoroquinolones: Levofloxacin, Moxifloxacin, and Ofloxacin; aminoglycoside injectables: Amikacin, Capreomycin, and Kanamycin; and other core second-line agents: Ethionamide, Bedaquiline, Clofazimine, Delamanid, and Linezolid. The critical concentrations were: 1.0 µg/ml for Amikacin, 2.5 µg/ml for Capreomycin, 2.5 µg/ml for Kanamycin, 1.0 µg/ml for Levofloxacin, 0.5 µg/ml for Moxifloxacin, 2.0 µg/ml for Ofloxacin, 5.0 µg/ml for Ethionamide, 1.0 µg/ml for Bedaquiline, 1.0 µg/ml for Clofazimine, 0.06 µg/ml for Delamanid and 1.0 µg/ml for Linezolid (14).

The MGIT 960 system monitors these growth patterns and can automatically interpret results as susceptible or resistant. An isolate is defined as resistant if 1% or more of the test population grows in the presence of the critical concentration of the drug. *Mycobacterium tuberculosis* strain H37Rv was used as a sensitive control for the susceptibility testing.

Data analysis

Data were entered in Microsoft Excel 2022, checked for inconsistencies, promptly cleaned, and then exported to IBM SPSS Statistics for Windows, Version 26.0., Armonk, NY: IBM Corp. USA for analysis. Descriptive parameters were employed to explain clinical profiles and drug susceptibility patterns. Mean and standard deviation were used to depict continuous variables. Statistical significance was determined to estimate the precision at a 95% confidence interval.

Quality assurance

Standard operating procedures were strictly followed during sputum collection, transportation, processing, and laboratory analysis. The internal quality control of the GeneXpert MTB/RIF assay was validated using non-RR and known RR *M. tuberculosis* H37Rv strains stored at -20^{o}C in the laboratory. The sterility of the culture media was checked by incubating the whole media at 37°C for 48 hours, and the performance was checked by known drug-susceptible *M. tuberculosis* H37Rv reference strain, ATCC 27294. The sterility of sample processing reagents was checked by inoculating all reagents on BHI. Positive and negative controls were included in each batch of culture and DST. Sterile molecular- grade water and reagent control were used as a negative control, while H37Rv ATCC25177 was used as a positive control. All laboratory results were recorded in a separate logbook. Moreover, all specimens were handled with appropriate precautions at all times and opened only within the BSL2 system.

Results

Sociodemographic and clinical characteristics

Mycobacterium tuberculosis was isolated from 216 new cases that were confirmed to be positive for *M. tuberculosis* complex using the GeneXpert MTB/RIF assay. We excluded 60 patients from the analysis due to nonviability and contamination reasons. A total of 156 *Mycobacterium tuberculosis* complex isolates were successfully recovered using the gold standard BD BACTEC™ MGIT™ 960 automated liquid culture system and were subjected to drug susceptibility testing (Figure 1).

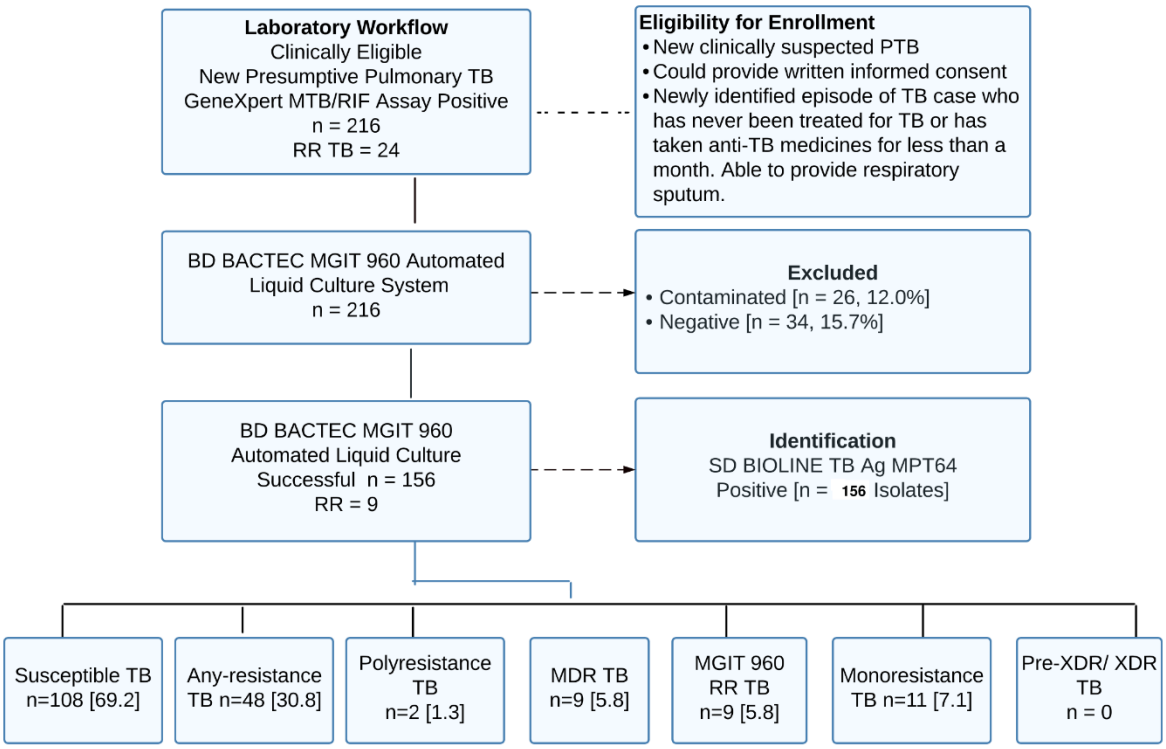

Figure 1. Flowchart showing enrollment eligibility and laboratory workflow

Among the study participants, males account for 53.8 % [84/156]. The median age of the study participants was 30.0 [range 6 – 75] years, with a mean and standard deviation of 33.56 and 12.65 years, respectively (Table 1).

Table 1. **Socio-demographic characteristics of study participants [n = 156]**

Variables Measured	Classification	Frequency Distribution	
		Number [n]	Percent [%]
Sex	Male	84	53.8
	Female	72	46.2
Age group	< = 29 Years	77	49.4
	30 – 50 Years	59	37.8
	> =51 Years	20	12.8
Marital status	Single	60	38.5
	Married	91	58.3
	Divorced	5	3.2
Residence	Urban	120	76.9
	Rural	36	23.1
Religion	Christian	130	83.3
	Muslim	26	16.7
Occupation	House Wife	23	14.8
	Daily Laborer	57	36.5
	Employed	52	33.3
	Unemployed	24	15.4
Monthly income in ETB[a]	< = 500	74	47.4
	501 – 1,999	33	21.2
	2,000 -10,000	49	31.4
Patients by health facility	SPH	100	64.1
	SulHC	25	16.0
	SenHC	31	19.9

ETB: Ethiopian Birr
SPH: Saint Peter Hospital
SulHC: Sululta Health Center
SenHC: Sendafa Health Center

Across all study participants, 17.3 % [27/156] had a previous history of confirmed PTB, 30.8 % [48/156] had a history of contact with active TB cases, 21.8% [34/156] had a treatment history for LTBI, and 16 % [25/156] had a history of BCG vaccination (Table 2).

Table 2. **Study participant clinical characteristics [n = 156]**

Variables Measured	Classification	Frequency Distribution	
		Number [n]	Percent [%]
Previous history of TB symptom	Yes	154	98.7
	No	2	1.3
History of contact with people with active TB	Yes	48	30.8
	No	108	69.2
History of any medicine	Yes	38	24.4
	No	118	75.6
History of BCG vaccination	Yes	25	16.0
	No	131	84.0
History of TB skin test	Yes	16	10.3
	No	140	89.7
History of TB IGRA test	Yes	20	12.8
	No	136	87.2
History of previous PTB+	Yes	27	17.3
	No	129	82.7
Treatment history for active or LTBI	Yes	34	21.8
	No	122	78.2
History of CXR for pulmonary TB	Yes	92	59.0
	No	64	41.0
History of chronic diseases	Yes	34	21.8
	No	122	78.2
Previous Rx and Dx history for CA	Yes	6	3.8
	No	150	96.2
History of HIV and IDUs	Yes	37	23.7
	No	119	76.3
History of hospital admission	Yes	24	15.4
	No	132	84.6
History of alcohol consumption	Yes	39	25.0
	No	117	75.0
History of khat consumption	Yes	30	19.2
	No	126	80.8
History of smoking	Yes	21	13.5
	No	135	86.5

TB: Tuberculosis; **BCG:** Bacille Calmette-Guerin; **IGRA:** Interferon-Gamma Release Assay; **PTB+**: Pulmonary TB Positive; **LTBI:** Latent TB Infection; **CXR:** Chest X-Ray; **Rx:** Treatment; **Dx:** Diagnosis; **CA:** Cancer; **HIV:** Human Immunodeficiency Virus; **IDUs:** Intravenous Drug Users.

Phenotypic DST and characteristics of isolates against first-line drugs

Overall, out of the 156 *M. tuberculosis* complex isolates, we found out that 14.1% [22/156] were resistant to at least one anti-TB drug, while 85.9% [134/156] of *M. tuberculosis* strains were pan-susceptible.

Further, 7.1% [11/156] of the isolates were mono-resistant *M. tuberculosis* strains, 5.8% [9/156] were identified as MDR-TB strains, and 3.8% [6/156] were resistant to all first-line anti-TB-drugs-that were tested. It is interesting to note that none of the isolates demonstrated monoresistance to RIF and EMB (Table 3).

Table 3. **Phenotypic drug susceptibility of first- and second-line anti-TB drugs [n = 156]**

Drug Profile and Regimen Category	Frequency Distribution		Type of Drug Resistance TB
	Susceptible n [%]	Resistant n [%]	
First Line Drug Profile			
All isolates with Resistance Profile	134 [85.9]	22[14.1]	
STM	142 [91.0]	14[9.0]	Any resistant TB
INH	138 [88.5]	18[11.5]	
RIF	147 [94.2]	9[5.8]	
EMB	149 [95.5]	7[4.5]	
STM+INH	155 [99.4]	1 [0.6]	
STM+INH+EMB	155 [99.4]	1 [0.6]	Poly-resistant TB
Total		**2[1.3]**	
INH	148 [94.9]	8 [5.1]	
STM	153 [98.1]	3 [1.9]	Mono-resistant TB
Total		**11[7.1]**	
INH+RIF	155 [99.4]	1 [0.6]	
INH+RIF+STM	154 [98.7]	2 [1.3]	MDR-TB
INH+RIF+STM+EMB	150 [96.2]	6 [3.8]	
Total		**9[5.8]**	
Second-Line Drug Profile			
MDR+AMK	22 [100]	0	
MDR+CAP	22 [100]	0	Pre-XDR-TB
MDR+KAN	22 [100]	0	
MDR+LFX	22 [100]	0	
MDR+MFX	22 [100]	0	
MDR+OFX	22 [100]	0	
MDR+BDQ	22 [100]	0	
MDR+CZF	22 [100]	0	
MDR+DLM	22 [100]	0	
MDR+LZD	22 [100]	0	
MDR+ETO	4[18.2]	18 [81.8]	
MDR+LFX+MFX+OFX	21 [100]	0	XDR-TB
MDR+LFX+MFX+BDQ+LZD	21 [100]	0	

STM: Streptomycin; **INH:** Isoniazid; **RIF:** Rifampicin**; EMB:** Ethambutol; **MDR:** Multidrug-resistant; **AMK:** Amikacin; **CAP:** Capreomycin; **KAN:** Kanamycin; **LFX:** Levofloxacin; **MFX:** Moxifloxacin; **OFX:** Ofloxacin; **BDQ:** Bedaquiline; **CFZ:** Clofazimine; **DLM:** Delamanid; **LZD:** Linezolid; **ETO:** Ethionamide**; TB:** Tuberculosis; **MDR-TB:** Multidrug-resistant tuberculosis; **XDR:** Extensively drug-resistant tuberculosis.

Phenotypic DST and characteristics of isolates against second-line drugs

Phenotypic DST against second-line anti-TB agents that include fluoroquinolones: Levofloxacin, Moxifloxacin, and Ofloxacin; aminoglycosides: Amikacin, Capreomycin, and Kanamycin; and other core second-line agents: Ethionamide, Bedaquiline, Clofazimine, Delamanid, and Linezolid were performed using BD BACTEC^{TM} MGIT^{TM} 960 instrument system. Among all drug-resistant strains (any), 81.8% [18/22] of isolates were resistant to ETO. However, all *M. tuberculosis* isolates that demonstrated any resistance to at least one first-line anti-TB drugs tested were found susceptible to WHO-recommended and nationally endorsed second-line anti-TB drugs (Table 3).

RR-TB detected in GeneXpert MTB/RIF assay Vs. BD BACTEC MGIT 960 liquid culture system

In this study, the rate of RR-TB detected was 10.9% [17/156] and 5.8% [9/156] using the GeneXpert MTB/RIF assay and the BD BACTEC^{TM} MGIT^{TM} 960 SIRE liquid culture system, respectively. Furthermore, rifampicin resistance detected using the GeneXpert MTB/RIF assay compared to the BD BACTEC^{TM} MGIT^{TM} 960 SIRE liquid culture system for automated DST showed a sensitivity of 100%, specificity of 94.6%, positive predictive value (PPV) of 52.9%, and negative predictive value (NPV) of 100% (Table 4).

Table 4. **Comparison of RR-TB detection rate between GeneXpert MTB/RIF Assay and BACTEC ™ MGIT™ 960 automated liquid culture system**

Measurement	Performance Rate [%]	95% CI
Sensitivity	100	[66.4 – 100]
Specificity	94.6	[89.6 – 97.6]
PPV	52.9	[36.5 – 68.8]
NPV	100	.
Accuracy	94.9	[90.2 – 97.8]
Kappa Value	0.667	.

PPV: Positive Predictive Value.
NPV: Negative Predictive Value.
CI: Confidence Interval.

Discussion

In this study, we identified a rate of 14.1% resistant strains of *M. tuberculosis* to at least one first-line anti-TB drug in new pulmonary TB cases, while 85.9% were found to be pan-susceptible. Previous studies reported comparable results in Ethiopia (15, 16), Germany (17), and Pakistan (18). However, a higher rate of resistant strains of *M. tuberculosis* was reported in Ethiopia (19), Zambia (20), Nepal (21), China (22), Kenya (23), and Myanmar (24). The possible reason for these varied rates of drug-resistant strains of *M. tuberculosis* reported among studies in the same as well as different geographic regions may be due to variations in study design, study period, health system efficiency to TB control activities, immunological and economic status of case and virulence factors of the Mycobacterial strain circulating in the community.

The rate of MDR-TB identified in this study was 5.8%, and this was congruent with the previous studies reported in Ethiopia (15,19) and Kenya (23). However, a higher rate of MDR-TB was reported in the previous studies in Ethiopia (25), Pakistan (18), Zambia (20), Nepal (21), China (22, 26), and Myanmar (24); and a lower rate of MDR-TB was reported in previous studies in Ethiopia (27) and Germany (17). Notwithstanding this observation, the WHO estimate of 1.1% MDR-TB in new TB cases disclosed in the 2022 global tuberculosis report (7) was much lower than the findings demonstrated in this study. The plausible assertions for the higher rate of MDR-TB among new pulmonary TB cases in our study than in the previous studies may reflect the emergence and transmission of MDR-TB in Ethiopia remained a challenge and the battle against TB is far from over. In addition to the mutation rates in this geographic area, virulence variations, poor treatment adherence, and inefficient health system could have a significant influence on the development of MDR-TB.

In this study, 3.8% of isolates were identified as resistant to all first-line anti-TB drugs tested. This proportion of resistance was higher than what was reported in a previous study (19) in Ethiopia. In addition, 7.1% of isolates were mono-resistant *M. tuberculosis* strains, and the highest monoresistance isolates to first-line anti-TB drugs were from INH [5.1%] and STM [1.9%]. A congruent monoresistance rate was reported in a previous study (28) in seven high TB-burden countries. This rate of INH and STM monoresistance respectively was lower than what was reported in a previous study (29) in Ethiopia. However, compared to our study findings, variably a higher rate of INH and a lower rate of STM monoresistance were reported in a previous study (20) in Zambia.

This study further demonstrated that the rate of resistance to any of the first-line anti-TB drugs is 9.0%, 11.5%, 5.8%, and 4.5% for STM, INH, RIF, and ETM respectively. A comparable rate of resistance was reported in a previous study (30) in Ethiopia, and an incomparably higher rate of resistance to INH and RIF was reported in a previous study (21) in Nepal. Interestingly, monoresistance to RIF and EMB was not demonstrated in all isolates in this study. The absence of monoresistance against RIF and EMB was reported in the previous studies (15, 16) in Ethiopia. A higher rate of sensitivity to RIF alone identified in this study may be a good indicator of the success of the DOTs program in the study area. However, the higher rate of INH monoresistance observed in this study is critical as INH is the main monotherapy drug used to treat LTBIs and the main chemoprophylaxis drug used in immunosuppressed TB cases in Ethiopia.

The findings of this study demonstrated that all MDR-TB isolates were susceptible to all recommended second-line anti-TB drugs tested and none of the isolates were found to be Pre-XDR or XDR-TB. Similar results were reported in previous studies (15,16) in Ethiopia, however, a higher rate of resistance against second-line anti-TB drugs was reported in the previous studies in Ethiopia (31, 32) and in Zambia (20). The absence of resistance to second-line anti-TB drugs in this study may reflect that the existing treatment of MDR-TB cases in the study area was encouragingly effective. This observation is critical owing to the implication that initiation of second-line drugs without initial DST might not be beneficial to the patient. Evidence from previous studies showed that diagnosed, untreated, and inappropriately managed MDR-TB contributes to a continuously sustained high prevalence of drug-resistant *M. tuberculosis* strains circulating in the community (33). However, a significant proportion [81.8%] of isolates exhibited resistance to Ethionamide in this study. Compared to

this study finding, a high rate of resistance against Ethionamide was reported in the previous studies in Kenya (34), Cambodia (35), and China (36), however, a lower rate of resistance to Ethionamide was also reported in China (37). The higher rate of resistance to Ethionamide observed in this study may be explained by the fact that Ethionamide and isoniazid do have a similar structural analog sharing common pathways that lead to cross-resistance (38, 39).

Although the primary aim of this study was not to validate rapid molecular diagnostics against the reference standard method, we compared the performance of GeneXpert MTB/RIF assay with BD BACTEC^{TM} MGIT^{TM} 960 SIRE liquid culture system related to RR-TB detection. Accordingly, the sensitivity of 100% GeneXpert MTB/RIF assay in detecting RR-TB in our study was in line with previous studies reported in Egypt (40), Bangladesh (41), Nepal (42), and Belgium (43), but higher than the sensitivity reported in China (44) and Brazil (45). Whereas, the specificity of 94.6% GeneXpert MTB/RIF assay in detecting rifampicin resistance in our study was lower than previous studies reported in Egypt (40), Bangladesh (41), Nepal (42), Belgium (43), and Brazil (45), but higher than the specificity reported in China (44). In this study, 52.9% of cases showed concordance between GeneXpert MTB/RIF assay and phenotypic DST in detecting RR-TB. However, a significant proportion, 47.1% of discordance rate was observed in this study. This result was lower than the concordance rate reported in Brazil (45), but comparable with what was reported in the previous studies in New Zealand (46) and Rwanda (47). Taking all together, the comparable sensitivity and inconsistent specificity may be due to limitations of the rapid molecular assay explained in previous studies as the presence of silent mutations in the *rpoB* gene (48), the presence of mutation outside of the RRDR of the rpoB gene (49) and low bacterial DNA in the clinical specimens contribute to the increased false rifampicin resistance (50). However, while GeneXpert MTB/RIF assay plays a critical role in the screening of *M. tuberculosis* in clinical specimens, its efficiency in identifying resistance against rifampicin introduces an unannounced high false positivity rate leading to a significant number of cases to be placed erroneously on a second-line anti-TB treatment regimen.

This study has certain limitations that need to be noted. Firstly, the study was conducted in new pulmonary TB cases and this finding could not be generalized to other types of TB disease classification. Secondly, we were not able to include PZA susceptibility testing due to shortages of validated and commercially available kits. Thirdly, we were unable to follow patients to look into treatment outcomes due to logistic reasons.

Conclusion

The rate of MDR-TB among new pulmonary TB cases remained high at fivefold the national and nearly twofold the global estimated rate. The rate of monoresistance against anti-TB drugs was also high. The absence of resistance against recommended second-line anti-TB drugs was quite encouraging. However, the high rate of resistance against Ethionamide would mean that its inclusion in the regimen may not have therapeutic benefit in this geographic area. Furthermore, the use of GeneXpert MTB/RIF assay alone for the detection of *M. tuberculosis* might introduce 15.7% false positivity and 47.1% false rifampicin resistance leading patients erroneously assigned in the MDR-TB category and placing on an unnecessary second-line anti-TB-treatment regimen. Initial drug susceptibility testing is critical from both patient and program perspectives before initiation of treatment with second-line anti-tuberculosis drugs. Furthermore, an enhanced and progressive validation of rapid molecular diagnostics against reference methods is recommended to improve patient outcomes.

Ethical considerations and approvals

Ethical approval was granted (Protocol No. IRB.039.2019) by the Institutional Review Board of the Department of Microbial, Cellular, and Molecular Biology, Faculty of Life Sciences, College of Natural and Computational Sciences, Addis Ababa University. This study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Written informed consent was obtained from each study participant and guardian of the children aged < 18 years. Data were delinked from the source file and analyzed in aggregates, and any illustrations avoided study subject identifiers to ensure anonymity.

Acronyms and Abbreviations

AMK: Amikacin; **BDQ:** Bedaquiline; **CAP:** Capreomycin; **CFZ:** Clofazimine; **CS:** Cycloserine; **DLM:** Delamanid; **DOT:** Directly-observed therapy; **DST:** Drug susceptibility testing; **EMB/E:** Ethambutol; **ETO:** Ethionamide; **FLDs:** First-line drugs; **FQ:** fluoroquinolone; **INH/H:** Isoniazid; **HH:** High-dose isoniazid; **KAN:** Kanamycin; **LFX:** Levofloxacin; **LZD:** Linezolid; **MDR:** Multidrug-resistant; **MDR-TB:** Multi-drug resistant tuberculosis; **MFX:** Moxifloxacin; **MTB:** *Mycobacterium tuberculosis* ; **MTBC:** *Mycobacterium tuberculosis* complex; **OFX:** Ofloxacin; **RIF/R:** Rifampicin; **RR-TB:** Rifampicin resistant tuberculosis; **SLDs:** Second-line drugs; **S/STM:** Streptomycin; **TB:** Tuberculosis; **XDR:** Extensively drug-resistant tuberculosis and **PZA/Z:** Pyrazinamide.

Consent for publication

Not applicable.

Data sharing and availability

All original data supporting this study's findings are available within the manuscript. However, additional data could be obtained from the corresponding author following a reasonable written request.

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University, Addis Ababa, Ethiopia, Ethiopian Public Health Institute's National TB Reference Laboratory, Addis Ababa, Ethiopia, and St. Paul's Hospital Millennium Medical College in Addis Ababa, Ethiopia.

Author contributions

GTA designed and executed the study, lead the manuscript writing, and was the coordinator of the study including all correspondences. BT and BP contributed to the conceptualization, design, and supervising of all activities. WS, MA, GD, MG, and BZ contributed to the microbiological culture and drug susceptibility testing, facilitating data management and manuscript writing. GTA, BT, and BP contributed to data analysis and were responsible for data visualization. GT drafted the initial manuscript. BT and BP critically reviewed all versions of the manuscript. All authors read and approved the final manuscript.

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Disclosure of interest

All authors declare that they have no competing interests to disclose in this work.

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