

First-Line Anti-Tuberculosis Drug Resistance and Pattern among Smear or GeneXpert Positive Pulmonary Tuberculosis Patients in Bale Zones of Oromia Region, Southeast Ethiopia

Beker Feto¹, Adunga Tsehay³, Bizuneh Belachew³, Mekdes Alemu³, Gemechu Guddisa⁴, Rawleigh Howe³, Lemessa Oljira², Adane Mihret³, Kidist Bobosha³, Abraham Aseffa³ and Berhanu Seyoum³

¹School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, Ethiopia

²School of Public Health, College of Health and Medical Sciences, Haramaya University, Ethiopia

³Armauer Hansen Research Institute, Addis Ababa, Ethiopia

⁴Adama Public Health Research and Referral Laboratory Center, Ethiopia

Background: Tuberculosis is a communicable disease that significantly contributes to global morbidity and ranks among the top causes of mortality worldwide. The problem is worsened by the emergence and spread of drug-resistant TB, especially in high-burden countries like Ethiopia. However, insufficient data on the magnitude and patterns of TB drug resistance, particularly in remote areas within these countries, hampers disease control. Therefore, this study aimed to determine the magnitude of the rate of resistance and resistance patterns to first-line anti-TB drugs among smear or GeneXpert-positive pulmonary tuberculosis patients visiting health facilities in selected districts of Bale Zones, Southeast Ethiopia.

Methods: An institution-based cross-sectional study was conducted in health facilities across eight districts of the Bale zones, including 152 smear- or GeneXpert-positive pulmonary tuberculosis patients with successful phenotypic drug susceptibility test results. Data on socio-demographics, TB treatment history, and other relevant variables were collected through a structured, pretested questionnaire. Mycobacterial drug susceptibility testing for first-line anti-TB drugs (rifampicin, isoniazid, streptomycin, and ethambutol) was performed using the indirect proportion method. Data analysis was conducted using SPSS version 20 to identify determinants of TB drug resistance.

Results: Of the 173 study participants, 152 were recruited, yielding a response rate of 88%. More than half of the participants were male (53.9%), with an age range of 5-65 years and a mean of 28.1 years (SD \pm 11.65). Resistance to at least one first-line anti-TB drug was 24.3% (95% CI: 17.8%-32%). Primary and secondary resistance were 24.1% (95% CI: 17.4%-31.9 %) and 28.6% (95% CI: 3.7%, 71 %), respectively. Rifampicin resistance was 1.3% (95% CI: 0.2%, 4.7%). Streptomycin exhibited the highest level of primary resistance (15.2%), followed by ethambutol (9%) and isoniazid (7.6%). Primary mono-resistance was greatest for streptomycin (9%) and ethambutol (4.7%). Multidrug-resistant TB was found in a single case, with resistance to all first-line agents. Resistance rates were not substantially different by sex, age, residence place, educational status, history of previous TB treatment, and history of imprisonment, history of chronic diseases or alcohol drinking.

Conclusion: A high magnitude of primary resistance to at least one first-line anti-TB drug and primary resistance to streptomycin was observed in Bale. Although the frequency of MDR TB was relatively low, resistance to Ethambutol and Isoniazid was common. Expanding district-level access to drug susceptibility testing facilities and strengthening existing are essential for establishing an effective monitoring system for tuberculosis drug resistance. This will enable the effective combat of tuberculosis and provide evidence-based treatment in underserved, remote settings.

Keywords: Tuberculosis, drug resistance, first-line drugs, Pattern, Bale

How to cite: Feto, B., Tsehay, A., Belachew, B., Alemu, M., Legasse, H., Guddisa, G., Howe, R., Oljira, L., Mihret, A., Bobosha, K., Aseffa, A., and Seyoum, B. 2024. First-Line Anti-Tuberculosis Drug Resistance Magnitude and Pattern among Smear or GeneXpert Positive Pulmonary Tuberculosis Patients in Bale Zones of Oromia Region, Southeast Ethiopia, *East African Journal of Health Biomedical Sciences*, Volume 8 (2): 27- 42

Introduction

Tuberculosis (TB) is a communicable disease that significantly contributes to global morbidity and ranks among the top causes of mortality worldwide, and caused an estimated 1.25 million deaths in 2023 (WHO, 2024b). An estimated 10 million people fell ill with TB, and it was the leading cause of death from a single infectious agent globally in 2018 ranking

above HIV/AIDS (WHO, 2019). It remains a major global health threat, causing an estimated 1.6 million deaths in 2021. Despite previous declines, the TB incidence rate rose by 3.6% between 2020 and 2021, reversing decades of progress (WHO, 2022). It was the world's second leading cause of death from a single infectious agent, after COVID-19 in 2022 (WHO, 2023a), and returned to being the world's leading cause of death from a single infectious agent in 2023 (WHO, 2024b). This increase is attributed to



disruptions in the provision of and access to essential TB services caused by the COVID-19 pandemic and other factors (WHO, 2022).

In Ethiopia, TB continues to be a major public health concern, placing the country among the 30 high TB burden and 30 high TB/HIV burden countries in the world and claiming the lives of thousands every year. It caused 21,000 deaths with an uncertainty interval (UI) of 13000-31000 deaths in HIV negatives and 1,700 (1100 - 2300) in HIV positives in 2022 (WHO, 2023a). The total TB incidence best estimate of Ethiopia, was 126 (UI: 85-176) per 100,000 population in 2022 (WHO, 2023a).

According to the Oromia regional health bureau's report, the number of pulmonary tuberculosis (PTB) cases was 29,895 (18,381 smear-positive pulmonary tuberculosis (SPPTB) and 11514 smear-negative pulmonary tuberculosis (SNPTB)) from July 2018 to June 2019 (OHB, 2024). The number increased to 39,820 (25,646 SPPTB) from July 2022 to June 2023 in the region, with an increasing trend from July 2019 through June 2022. Moreover, the number of pulmonary TB cases in the two Bale zones was 1339 (849 SPPTB and 490 SNPTB) from July 2018 to June 2019, and became 1,936 (1,310 SPPTB and 626 SNPTB) from July 2022 to June 2023, with an increasing trend from July 2019 through June 2022 (OHB, 2024). In addition, the number of relapse cases in these zones was 60 from July 2018 to June 2019, 35 from July 2019 to June 2020, and 48 from July 2022 to June 2023 (OHB, 2018, 2024).

The challenge of TB is further complicated by the rise and spread of drug-resistant TB (DR-TB), particularly multidrug-resistant tuberculosis (MDR-TB). This necessitates treatment with second-line regimens, which are more complex than those used for the treatment of non-drug-resistant TB patients (Shah *et al.*, 2007). In Ethiopia, the number of incident MDR/RR TB cases was 1,400 (970-2000) in 2019 (WHO, 2020a) and 2 000 (1,200 – 2,800 in 2022 (WHO, 2023b) with the MDR/RR TB incidence rate of 1.3 (0.87–1.8) in 2019 (WHO, 2020a) and 1.6 (1-2.3) in 2022 (WHO, 2023b). Hence, drug-resistant TB is also a concern to the national TB control program of Ethiopia, and poses a threat to its TB control efforts. Knowledge of the drug resistance magnitude and pattern of TB in the country, particularly in its remote areas with higher TB burden,

is essential to set evidence-based improvement of treatment regimens to mitigate this health threat; ultimately to end TB. Unfortunately, in resource-limited countries like Ethiopia, particularly in its peripheral areas, data on the magnitude of the rate of TB drug resistance and resistance patterns are rarely available.

In the two Bale zones, the number of reported MDR cases showed considerable fluctuation across the reporting periods: seven cases were recorded between July 2017 and June 2018, one case between July 2018 and June 2019, none between July 2019 and June 2021, one case between July 2021 and June 2022, and none between July 2022 and June 2023 (OHB, 2018, 2024). Despite higher magnitudes of SPPTB and relapse cases in the two Bale Zones, the reported number of MDR seems low. Regarding the drug susceptibility testing (DST) service in these zones, the majority of districts do not have any facility to run DST, culture, or DNA-based tests. Even though there are four districts that do have hospitals that provide rifampicin resistance test with GeneXpert, their service is very intermittent and hardly accessible to the remote districts. Accordingly, the low number of reported MDR cases over the years may not accurately reflect the true magnitude of drug resistance in these areas. Accordingly, the current drug-resistance status of tuberculosis (TB) in the Bale zones remains largely unknown. This knowledge gap raises concern about ongoing transmission and the potential emergence of drug-resistant TB within the community, which could result in more difficult-to-treat cases and increased morbidity and mortality. Hence, this study was conducted to determine the magnitude of drug resistance rate and resistance pattern of TB to first-line anti-TB drugs in the Bale zones of Oromia region, southeastern Ethiopia. The findings of this study will help the local TB control program (other stakeholders) to implement evidence-based interventions to control the spread and emergence of drug-resistant TB in Bale.

Materials and Methods

Study Setting, Design, and Period

A facility-based cross-sectional study was conducted from June 2021 to December 2022 among eight districts of the Bale Zones, Oromia, Ethiopia to establish the magnitude and drug resistance pattern of TB to first-line anti-TB medications. Bale Zone had a total population of 1,840,746 in 2017, of which 269,139

lived in urban settings (CSAE, 2013). The zone has about 84 health centers and five hospitals that offer Directly Observed Treatment, Short-course (DOTS) for TB (BHO, 2017). The study targeted six main health facilities that provide TB diagnosis and treatment services for eight districts out of 20: Ginnir Hospital (for Ginnir, Sewena, and Rayitu districts), Robe, Goba, Dello Mena, Mada Wolabu hospitals, and Angetu Health Center (for Harana Buluk district). Raytu and Sewena health centers were utilized on an as-and-when basis, with patients continuing to attend Ginnir Hospital when the services of that center were not available (Fig. 1).

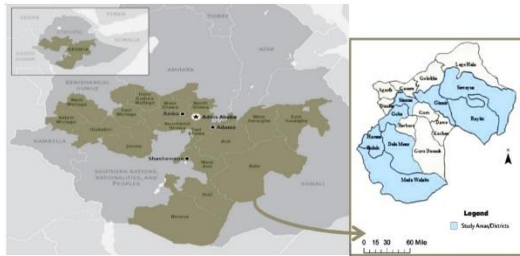


Figure 1: Map of Study Area

Population, Inclusion/ Exclusion Criteria

All smear or GeneXpert-positive pulmonary tuberculosis patients who had not started anti-TB treatment yet were included in the study. On the other hand, extrapulmonary TB cases, smear-negative cases where acid-fast staining (AFS) was used as a diagnostic tool, GeneXpert-negative cases where GeneXpert was used as a diagnostic tool, and smear or GeneXpert-positive pulmonary tuberculosis patients who had already started anti-TB treatment were excluded from the study.

Sample Size and Sampling Technique

The sample size was calculated using a single population proportion formula with finite population correction, in accordance with WHO guidelines for tuberculosis drug-resistance surveillance. (WHO, 2009). Estimation was done using a priori estimate of prevalence of rifampicin resistance of 1.7% (Seyoum *et al.*, 2014), precision 1%, 95% confidence interval, and 20% non-response rate. As 185 smear-positive cases had been noted in the eight study districts during 2017/2018 (OHB, 2018), the sample size was increased by 20% to compensate for potential losses, hence having a fi-

nal sample size of 173. Districts were chosen purposively to mirror the economic and geographical diversity of the area, including pastoral, agro-pastoral, and agrarian livelihoods, as well as highland and lowland. The participants eligible for the study were selected by taking those identified at the DOTS centers of the sampled Bale Zones health facilities consecutively.

Data Collection Instruments and Procedures

Data were collected through face-to-face interviews using a structured questionnaire to gather socio-demographic information, TB treatment history, and other relevant data at selected health facilities.

Sputum samples were collected after participants received instructions from laboratory technicians on proper sample collection. Samples were delivered in labeled, wide-mouthed, leak-proof, screw-capped 50 ml flacon tubes, checked for quality and quantity, and stored at 2–8°C for up to 7 days at the laboratories of selected health facilities. A total of 400 sputum samples from smear- or GeneXpert-positive patients were transported at +4°C under cold chain conditions to the Adama Public Health Research and Referral Laboratory Center for culture and further analysis. A total of 400 sputum samples were collected, assuming that at least 43.25% would yield growth during primary culturing and sub-culturing, to obtain a final sample size of 173 for the phenotypic drug resistance study.

Sputum processing and *M. tuberculosis* isolation were performed using the standard Petroff's method (NALC-NaOH), with centrifugation to concentrate organisms (Kent, 1985). Sediment was reconstituted in phosphate-buffered saline and inoculated on Löwenstein-Jensen (LJ) medium with 0.75% glycerol, incubated at 37°C for up to 8 weeks, and monitored weekly for growth. Acid-fast bacilli were confirmed by Ziehl-Neelsen staining for phenotypic drug susceptibility testing (pDST).

Drug susceptibility was tested using a modified indirect proportion method (van Klingeret *et al.*, 2007) on Middlebrook 7H10 agar supplemented with OADC and glycerol. Four first-line drugs (rifampicin, isoniazid, streptomycin, ethambutol) were tested at critical concentrations of 1 µg/ml, 0.2 µg/ml, 2 µg/ml, and 5 µg/ml respectively. Bacterial suspensions were adjusted to McFarland standard 1.0. Controls included drug-free wells with diluted and undiluted bacterial suspensions. Strains showing less than 1% growth compared to controls were classified as susceptible;

those exceeding 1% were resistant. Contaminated or borderline tests were repeated (Wedajo *et al.*, 2014).

Operational Definition/Definition of Terms

Drug-resistant TB (DR-TB) broadly refers to TB caused by strains resistant to any tested TB medication. Mono-resistant TB is defined as resistance to a single TB drug. Poly-resistant TB involves resistance to at least two TB drugs, excluding both isoniazid and rifampin. Multidrug-resistant TB (MDR-TB) is characterized by resistance to at least isoniazid and rifampin, the two most effective first-line drugs. The term MDR/RR-TB includes both MDR-TB and rifampicin-resistant TB (RR-TB). Primary resistance occurs in individuals who have never received TB treatment. Secondary resistance appears in those with a history of prior TB therapy (WHO, 2013).

Based on their history of previous treatment for tuberculosis, a person with TB disease is classified as follows: a new case refers to an individual who has never been treated for TB or has taken TB medications for less than one month; a previously treated case or recurrent case refers to an individual who was previously treated for TB, declared cured or had completed treatment at the end of their most recent course, and is now diagnosed with a new episode of TB (WHO, 2024a).

Data Processing and Analysis

Data collected from smear- or GeneXpert-positive participants via questionnaire, along with phenotypic drug susceptibility test (pDST) results, were entered using REDCap and analyzed with SPSS version 20. The data were cleaned, organized, and presented in tables and figures. Overall, primary and secondary resistance levels to first-line anti-TB drugs (rifampicin, isoniazid, streptomycin, and ethambutol) were measured. Descriptive statistics compared variables between drug-susceptible and drug-resistant patients. Drug resistance was assessed on a binary scale as either resistant or susceptible for each tested drug.

Data Quality Control

Sputum samples of good quality and sufficient volume (5 ml thick sputum containing mucoid or muco-purulent material) were collected in sterile screw capped Falcon tubes and stored at 2–8°C refrigerator until

transported. All samples were transported to Adama public health research and referral laboratory center using a cold chain system (40 °C), and processed at arrival with a negative control included for each batch. The *M. tuberculosis* reference strain H37Rv (ATCC 27294), which is sensitive to all conventional anti-tuberculosis medications, was employed as a negative control for drug resistance in each drug susceptibility testing (DST) assay. Additionally, the questionnaire used for the interview underwent a pretest before implementation, and trained data collectors collected the data under the close supervision of skilled supervisors.

Ethical Consideration

Ethical approval for the study was granted by the Haramaya University College of Health and Medical Sciences Institutional Health Research Ethics Review Committee (IHRERC) and the AHRI/ALERT research ethics review committees with Ref. NO. IHRERC/141 /2019) and Protocol Number: PO/09/19. Written informed consent from participants aged >18 years, informed consent from parents or guardians and assent from participants aged 12–17 years were obtained for minors. Data obtained was kept confidential and utilized solely for the study. Patients who were found to have drug-resistant TB were informed of their results through their respective health institutions and were accordingly treated. Health education about the prevention of disease transmission was also provided to patients and guardians.

Results

Socio-demographic characteristics of participants.

During the study period, sputum samples from 230 (57.5%) patients showed mycobacterial growth on LJ culture. For 152 of these patients, isolates were successfully recovered on subculture and had successful phenotypic drug susceptibility test results; and hence included in this study. Among the 152 study participants, 53.9% (82) were male, 60.5% (92) were farmers, and 77% (117) were rural residents. In addition, 53.3% (81) had no formal education, 4.6% (7) had a history of TB treatment, and 7.9% (12) had a history of chronic disease. Their age ranged from 5-65 years, with the mean age of 28.1 years $SD \pm 11.65$), and 58.6 % were in the age range of 15-29 years (Table 1).

Table 1: Demographic characteristics and previous history of TB treatment of Pulmonary Tuberculosis Patients in Bale Zone of Oromia, Southeast Ethiopia, 2022, (*n* = 152)

Characteristic	Frequency(n)	Percentage (%)
Sex		
Male	82	53.9
Female	70	46.1
Age Groups		
5 – 14	12	7.9
15 – 29	89	58.6
30 – 44	30	19.7
45 – 59	16	10.5
60 – 69	5	3.3
Educational status		
Formal education	71	46.7
No formal education	81	53.3
Occupational status		
Farmer	92	60.5
Student	23	15.1
Unemployed/no job	18	11.8
Merchant	12	7.9
Laborer	5	3.3
Civil Servant	2	1.3
Place of Residence		
Urban	35	23
Rural	117	77
Previous history of TB Treatment		
No	145	95.4
Yes	7	4.6
Chronic disease history		
No	140	92.1
Yes	12	7.9
Imprisonment history		
No	145	95.4
Yes	7	4.6
Alcohol drinking history		
No	148	97.4
Yes	4	2.6

Phenotypic DST and characteristics of isolates against first-line anti-TB drugs

From the total 152 culture-positive pulmonary TB patients, 145 (95.4 %) were new, whereas 7 (4.6 %) were previously treated patients with first-line anti-TB drugs. Overall, from the 152 mycobacterial isolates, 24.3% (37/152) (CI: 17.8%, 32%) of them were found resistant to at least one first-line anti-TB drug. From the 145 isolates of new TB cases, 24.1% (35) (95% CI: 17.4%, 31.9%) of them were found resistant to at least one first-line anti-TB drug (primary resistance), and 2 of the 7 isolates from previously treated TB cases were resistant to at least one of first-line anti-TB drugs (Table 2). Resistance to any first-line anti-TB drug among new cases was 15.2% (22) (95% CI: 9.8% -22.1%) for streptomycin (STM), 9% (13) (95% CI:

4.9% - 14.8%) for ethambutol (EMB), 7.6% (11) (95% CI: 3.8%,13.2%) for isoniazid (INH) and 0.7% (1) (95% CI: 0.01% – 3.8%) for rifampicin (RIF). In fact, 17.2% (25) of new isolates were mono-resistant *M. tuberculosis* isolates. From these mono-resistant strains, 9% (13), 4.8% (7), 2.8% (4), and 0.7% (1) of them were STM, EMB, INH, and RIF mono-resistant, respectively. On the other hand, there was only streptomycin mono-resistance among previously treated patients, accounting for 14.3 % (1) resistance (Table 2). Moreover, 6.9 % (10) of the *M. tuberculosis* isolates were poly-resistant to first-line anti TB drugs: 2.8 % (4) for INH and STM, 2.1 % for ETB and STM, 1.4 % (2) for INH, ETB and STM, and 0.7 % (1) for INH and EMB (Fig. 2). There was only one MDR (0.7%) among the 152 isolates and it was from previously

treated patients and was resistant to all four first-line anti TB drugs. Hence, the secondary MDR was 14.3%

(1), and it was the only case with MDR and the only case resistant to all first-line anti-TB drugs tested.

Table 2: Resistance Pattern to First-Line Anti-Tuberculosis Drugs among Pulmonary Tuberculosis Patients in Bale Zone of Oromia, Southeast Ethiopia, 2022 ($n = 152$)

Resistance	Total case n = 152)		New Cases (n = 145)		Previously Treated Cases (n =7)	
	No	%	No	%	No	%
Susceptible	115	75.7	110	75.9	5	71.4
Any resistance to at least one anti-TB drug	37	24.3	35	24.1	2	28.6
Any resistance						
INH	12	7.9	11	7.6	1	14.3
RIF	2	1.3	1	0.7	1	14.3
EMB	14	9.2	13	9	1	14.3
STM	24	15.8	22	15.2	2	28.6
Mono – Resistance						
INH only	4	2.6	4	2.8	-	-
RIF only	1	0.66	1	0.7	-	-
EMB only	7	4.6	7	4.8	-	-
STM only	9	8.6	13	9	1	14.3
Total Mono – Resistance	25	16.4	25	17.2	-	-
MDR	-	-	-	-	1	14.3
Poly – Resistance						
INH & STM	-	-	4	2.8	-	-
EMB & STM	-	-	3	2.1	-	-
INH, EMB & STM	-	-	2	1.4	-	-
INH & STM	-	-	1	0.7	-	-
Total poly – Resistance	-	-	10	7	-	-

R: rifampicin, H: isoniazid, S: streptomycin, E: ethambutol, INH: isoniazid, STP: streptomycin, EMB: ethambutol

Discussion

Among 152 culture-positive pulmonary TB patients, 95.4% were newly diagnosed and 4.6% had prior treatment with first-line anti-TB drugs. Overall, 24.3% of isolates showed resistance to at least one first-line drug, with a 24.1% resistance rate among new cases (primary resistance) and a 28.6% resistance rate among previously treated cases (secondary resistance). Among new cases, the highest resistance to any first-line anti-TB drug was observed for streptomycin (15.2%), followed by ethambutol (9%), isoniazid (7.6%), and rifampicin (0.7%). Mono-resistance was observed in 17.2% of isolates from new cases, predominantly to streptomycin (9%), while 6.9% exhibited poly-resistance involving combinations of INH, EMB, and STM. Only one MDR case (0.7%) was identified, originating from a previously treated patient and resistant to all four first-line drugs.

In this study, the 24.3% overall resistance among new and previously treated TB patients to at least one first-line anti-TB drug is lower than the 34.1% rate reported in Northwestern part of Ethiopia (Seid, 2023), 33.4% of Ethiopian national survey (Getahun *et al.*, 2015), the pooled rate from Sudan (47%) (Hajissa, 2021) and 42% from Nigeria (Otu, 2013). It is comparable to the rate reported in the Northwestern part of Ethiopia (29.2%) (Yigzaw WB, 2021), central Ethiopia (21.7%) (Hamusse *et al.*, 2016), and Zambia (23.5%) (Monde, 2023) but higher than the rate (percentage) reported in northwest Ethiopia (16.1%) (Lobie *et al.*, 2020) and Central and Southern Ethiopia (14.4%) (Tilahun *et al.*, 2023).

According to our current findings, the primary resistance to at least one first-line anti-TB drug was 24.1% (95% CI: 17.4% - 31.9%). This rate is lower than the rate observed in the Eastern part of Ethiopia

(32.5%) (Mitike *et al.*, 1997) but higher than the rates observed in central Ethiopia (15.3%) (Hamusse *et al.*, 2016) and comparable to the rate observed in southwestern Ethiopia (18.4%) (Abebe *et al.*, 2012) and central Ethiopia (22.2%) (Bedewi Z, 2017). The 28.6% (95% IC: 3.7% -71%) rate of secondary resistance observed in this study is comparable to the rate in central Ethiopia (48.8%) (Hamusse *et al.*, 2016). The differences in overall, primary, and secondary drug resistance rates to at least one first-line anti-TB drug between our study and others could be attributed to variations in sample size, study populations, treatment adherence, and disparities in healthcare facilities (including the availability and quality of health services, access to anti-TB drugs, presence of trained healthcare providers, and support for treatment adherence).

Moreover, this study has shown that there are high rates of resistance of TB to at least one first-line anti-TB; the majority of the resistance was primary (24.1%). A primary drug resistance rate of this magnitude has profound implications for tuberculosis prevention and control programs. That is, the first-line anti-TB drugs might be less effective, and this in turn can lead to higher chances of treatment failure, emergence and spread of resistant TB strains within communities (Federal Ministry of Health, 2018). Accordingly, the TB control program (local, regional, and national stakeholders in collaboration with international bodies) needs to prioritize availing a rapid drug susceptibility testing (DST) facility at the district level to ensure that TB patients have access to timely DST to identify drug resistance early and initiate appropriate treatment. Moreover, engaging communities in TB (DR-TB) control efforts through outreach educational campaigns might help to reach the remote rural communities of the Bale zones, and hence would contribute much to fighting against this public health problem.

The resistance rate against STM (15.2%; 95% CI: 9.8%, 22.1%) is higher than the rates observed in Southwestern Ethiopia (8.1%) (Abebe *et al.*, 2012), Kenya (6.25%) (Kerubo *et al.*, 2016) and comparable to the pooled rate from Sudan (22.1%) (Hajissa *et al.*, 2021) but was lower than the rate from the Ethiopian national TB survey (26.6 %) (Getahun *et al.*, 2015). The elevated prevalence of STM resistance is a long-

term outcome of its historical use as a monotherapy, before the adoption of combination regimens involving more effective antibiotics ((Rocha *et al.*, 2021). Though, STM is not currently included in the Ethiopian national TB treatment protocol as a first-line anti-TB drug, the widely spread STM resistance at a national level and in our study area is worrisome, as there is a hypothesis that states the STM-resistant strains that are highly transmissible and possibly more prone to acquire other drug resistance (Röcha *et al.*, 2021). As a result, exploring the role of STM resistance in controlling the spread of existing and the development of new drug-resistant TB seems very important, and hence should not be neglected.

Ethambutol resistance was the second most frequent, with 9% (95% CI: 4.9% - 14.8%) rate of any resistance and 4.8% (95% CI: 2.0% - 9.7%) mono resistance in our study. Lower EMB mono-resistance rates have been observed in different parts of Ethiopia: Central Ethiopia (1.5%) (Bedewi Z, 2017) and Eastern Ethiopia (0.3%) (Seyoum *et al.*, 2014). On the other hand, comparable rates have been reported in Southwestern Ethiopia (1.5%; 95% CI: 0.4–5.2) (Abebe *et al.*, 2012), Sudan (2.1%) (Hajissa *et al.*, 2021), and Kenya (4.4%) (Sitienei *et al.*, 2017). Obviously, there is a higher rate of EMB drug resistance in the current study area than in other parts of the country. This magnitude of resistance in Bale zones is worrisome and might have detrimental consequences. This is because EMB is being used as the first-line anti-TB drug regimen in Ethiopia, and ethambutol is incorporated into the initial phase of the standard treatment regimen for tuberculosis presumed to be drug-susceptible, serving as a protective agent against possible unrecognized resistance to the three core medicines: rifampin, isoniazid, and pyrazinamide (Horsburgh CR Jr, 2015). In this remote area, where drug susceptibility testing service is limited and the resistance status of patients to these core anti-TB drugs remains unknown, this higher rate of ethambutol resistance could compromise the effectiveness of the entire TB treatment regimen.

In addition, a report of the WHO indicated that patients infected with the *Mycobacterium tuberculosis* strain and showing simultaneous resistance to EMB and INH or EMB and RIF had an increased risk of treatment failure and further acquired resistance (Falzon *et al.*, 2011). Moreover, EMB is also suggested as a potential

supplementary agent for a treatment regimen of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) TB (WHO, 2020c). Hence, Bale zone TB control stakeholders need to assess what went wrong, like patient adherence and whether the health institutions follow standard work guidelines to check for proper completion of the treatment regimen by every patient. Moreover, periodic assessment of the drug susceptibility of Ethambutol might be essential to find an alternative medication if the problem persists.

The rate of any resistance against INH among new pulmonary TB patients in our study (7.6%: 95% CI: 3.8%, 13.2%) is comparable to the rates reported in central Ethiopia (9.4 %) (Hamusse *et al.*, 2016), southwestern Ethiopia (13.2%) (Abebe *et al.*, 2012), and to the rate from Ethiopian national survey (5.5%) (Getahun *et al.*, 2015) and Kenya (5.2%) (Sitienei *et al.*, 2017) but it is lower than the rates observed in North Ethiopia (13.4%) (Weleki-dan *et al.*, 2020) and pooled rate from Sudan (15.7%) (Hajissa *et al.*, 2021). Although higher rates of INH mono resistance among new pulmonary TB patients were reported from different parts of Ethiopia (6.3% - 9.5%) (Abebe *et al.*, 2012; Bedewi *et al.*, 2017; Lobie *et al.*, 2020; Seyoum *et al.*, 2014), the rate from the present study (2.7%) is higher than the rate from the Ethiopian national survey (1.1%) (Getahun *et al.*, 2015). In general, the primary rate of INH resistance in our study area is concerning, as INH is part of the current TB standard regimen and as its resistance has multiple implications. For example, studies have shown that resistance to INH reduces the probability of treatment success as well as increment of the risk of acquiring resistance to other important first-line drugs such as RIF. Moreover, the management of INH-resistant TB takes a longer time and hence contributes to a higher burden of tuberculosis (Alemu *et al.*, 2023; Manson *et al.*, 2017; Menzies *et al.*, 2009).

In our study, the primary resistance to RIF was 0.7% and only observed in only one isolate and manifested as mono resistance. Similar or slightly higher rates were observed in different parts of Ethiopia and national surveys of African countries: 0.7% in southwest Ethiopia (Abebe *et al.*, 2012), 1.7% in Eastern Ethiopia (Seyoum *et al.*, 2014), 1.9% in central Ethiopia (Bedewi Z, 2017), 0.5% in Kenya (Sitienei *et al.*, 2017), and 0.9% in Tanzania (Mutayoba *et al.*, 2022).

Similarly, this rate is in line with the estimated 2019 Ethiopian national percentage of new cases of TB with MDR/RR TB (0.71%) (WHO, 2020b) and a bit lower than the 2022 (1.1%) (WHO, 2023b). Moreover, the secondary resistance to RIF in this study was 14.3% (95% CI: 0.4%-57.9%), it is comparable to the 16% national estimate in 2019 (WHO, 2020b) and 12% in 2022 (WHO, 2023b).

Though, the magnitude of rifampicin resistance in our study area is in line with the national as well as regional values, it is still alarming. Because of its potency and role in preventing the development of drug resistance, rifampicin has been a cornerstone of TB treatment regimens. Hence, resistance to rifampicin can pose serious consequences in patient care and public health, such as difficulty in finding effective alternative TB treatment options, emergence and spread of multidrug-resistant TB, and increased mortality from MDR tuberculosis (Malenfant & Brewer, 2021; Prasad *et al.*, 2018).

The challenge of TB is complicated by the rise and spread of drug-resistant TB (DR-TB). Various risk factors, including a previous history of TB treatment, poor adherence during the first-line anti-TB treatment, contact with an MDR-TB, and being unemployed, are known to be associated with DR-TB. Identification of these factors in a given geographical setting is crucial in preventing and controlling DR-TB (Alemu A, 2022; Bedewi Z, 2017). On the other hand, this study found no statistically significant difference in the rate of resistance to at least one first-line anti-TB drug and the assessed risk factors. This might be due to a smaller sample size, which can be seen from the wider confidence intervals of our findings.

Strengths and Limitations

This study was conducted in an underserved, remote area of the Bale zones, and its findings can be used to inform evidence-based interventions by the tuberculosis control program. Moreover, the validity of data was maintained by using a standardized and pretested data collection tool, trained professional data collectors, and following standardized operational procedures. On the other hand, the sample size might have contributed to the lack of statistically significant difference in the rate of resistance to at least one first-line anti-TB

drug and the assessed risk factors, and this is considered a limitation of this study. Another limitation of this study was the inability to conduct pyrazinamide phenotypic drug susceptibility testing due to resource constraints. Including pDST would have offered a more complete picture of resistance to first-line anti-TB drugs in the Bale Zones.

Conclusion

A high magnitude of primary resistance to at least one first-line anti-TB drug and primary resistance to STM was observed in Bale. Although there was a relatively low magnitude of MDR-TB, the rate (percentage) of primary resistance to EMB and INH was found to be common. To effectively combat tuberculosis and provide evidence-based treatment, it is essential to develop a system that routinely monitors drug resistance status, at least for INH and EMB, in addition to rifampicin, within the Bale zones. This system will empower healthcare providers with crucial data to tailor treatments to patient needs. Expanding access to DST facilities at the district level and strengthening existing DST facilities are indispensable steps toward establishing a functional monitoring system for tuberculosis drug resistance patterns. Moreover, disseminating information through mass media (e.g., radio) about TB, DR/RR/MDR TB, and available services to the public, as well as conducting outreach educational campaigns, may help engage communities in remote rural areas of the Bale zones and significantly contribute to the fight against TB, DR/RR/MDR TB..

Author Contributions

All authors contributed substantially to the conception, study design, data analysis, and interpretation of the findings. They were also involved in drafting the manuscript, reviewing the initial version, and collectively agreed on the target journal for publication. Each author has read and approved the final version of the manuscript and accepts full responsibility for its content under all circumstances.

Acknowledgments

The authors gratefully acknowledge Haramaya University and AHRI for funding this study. We also extend our sincere appreciation to the Adama Public

Health Research and Referral Laboratory Center for their support with the laboratory work. Special thanks are due to the study participants, data collectors, and supervisors for their invaluable contributions in making this study possible.

Competing Interests

The author declares that they have no competing interests.

Funding Statement

This study was funded by Haramaya University, the Ethiopian Ministry of Education, and AHRI. The funding organizations had no involvement in the study design, data collection, analysis, interpretation of the results, or manuscript preparation.

List of Abbreviations

AFB: Acid-fast bacillus, AHRI: Armauer Hansen Research Institute, AIDS: Acquired Immuno-Deficiency Syndrome, ALERT: Africa Leprosy Tuberculosis Rehabilitation and Training, CI: Confidence Interval, CSA: Central statistical Agency, DOTS: Directly Observed Treatment, Short-course, DST: Drug Susceptibility Testing, EMB-ethambutol, HIV: Human Immune Deficiency Virus, INH: Isoniazid, LJ: Lowenstein Jensen, MDR TB: Multidrug Resistant Tuberculosis, MQ-H₂O: Milli-Q water, NALC-NaOH: N-acetyl-L-Cysteine–Sodium Hydroxide, OADC: Oleic Albumin Dextrose Catalase, pDST: Phenotypic Drug Susceptibility Testing, RIF: Rifampicin, RR – TB: Rifampicin-Resistant Tuberculosis, SNPTB: Smear Negative Pulmonary Tuberculosis, SPPTB: Smear Positive Pulmonary Tuberculosis, STM: Streptomycin, TB: Tuberculosis, WHO: World Health Organization, XDR TB: Extensively Drug-Resistant Tuberculosis.

References

- Abebe, G., Abdissa, K., Abdissa, A., Apers, L., Agonafir, M., de-Jong, B. C., et al. (2012). Relatively low primary drug-resistant tuberculosis in southwestern Ethiopia. *BMC research notes*, 5(1), 1-6.
- Alemu, A., Bitew, Z. W., Diriba, G., Seid, G., Moga, S., Abdella, S., et al. (2023). Poor treatment outcome and associated risk factors among pa-

- tients with isoniazid mono-resistant tuberculosis: A systematic review and meta-analysis. *PloS one*, 18(7), e0286194.
- Alemu A., B. Z., Diriba G., Gumi B. (2022). Risk factors associated with drug-resistant tuberculosis in Ethiopia: A systematic review and meta-analysis. *Transbound Emerg Dis*. 2022 Sep;69(5):2559-2572. doi: 10.1111/tbed.14378. Epub 2021 Nov 19. PMID: 34741434.
- Bedewi, Z., Mekonnen, Y., Worku, A., Medhin, G., Zewde, A., Yimer, G., et al. (2017). Mycobacterium tuberculosis in central Ethiopia: drug sensitivity patterns and association with genotype. *New microbes and new infections*, 17, 69-74.
- Bedewi Z, M. Y., Worku A, Medhin G, Zewde A, Yimer G, Pieper R, Ameni G. (2017). Mycobacterium tuberculosis in central Ethiopia: drug sensitivity patterns and association with genotype. *New Microbes New Infect*. 2017 Mar 3;17:69-74. Doi: 10.1016/j.nmni.2017.02.003. PMID: 28377803; PMCID: PMC5369855.
- BHO. (2017). Data from Bale Zone Health office, 2017, Oromia, Ethiopia.
- CSAE. (2013). Central Statistical Agency of Ethiopia. Population Projection of Ethiopia for All Regions at Wereda Level from 2014 – 2017. Addis Ababa, Ethiopia.
- Falzon, D., Jaramillo, E., Schünemann, H., Arentz, M., Bauer, M., Bayona, J., et al. (2011). WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. In: *Eur Respiratory Soc*.
- Federal Ministry of Health, E. (2018). National guidelines for management of TB, DR-TB, and leprosy in Ethiopia, Sixth Edition. <https://www.afro.who.int/publications/national-guidelines-tb-drug-resistant-tb-and-leprosy-ethiopia-sixth-edition>
- Getahun, M., Ameni, G., Kebede, A., Yaregal, Z., Hailu, E., Medih, G., et al. (2015). Molecular typing and drug sensitivity testing of Mycobacterium tuberculosis isolated by a community-based survey in Ethiopia. *BMC Public Health*, 15(1), 1-7.
- Hajissa, K., Marzan, M., Idriss, M. I., & Islam, M. A. (2021). Prevalence of drug-resistant tuberculosis in Sudan: A systematic review and meta-analysis. *Antibiotics*, 10(8), 932.
- Hamusse, S. D., Teshome, D., Hussen, M. S., Demissie, M., & Lindtjörn, B. (2016). Primary and secondary anti-tuberculosis drug resistance in Hitossa District of Arsi zone, Oromia regional state, Central Ethiopia. *BMC Public Health*, 16(1), 1-10.
- Horsburgh CR Jr, B. C. R., Lange C. (2015). Treatment of Tuberculosis. *N Engl J Med*. 2015 Nov26;373(22):2149-60. doi:10.1056/NEJMra1413919. PMID: 26605929.
- Kent, P. T. (1985). Public health mycobacteriology: a guide for the level III laboratory. US Department of Health and Human Services, Public Health Service, Centers.
- Kerubo, G., Amukoye, E., Niemann, S., & Kariuki, S. (2016). Drug susceptibility profiles of pulmonary Mycobacterium tuberculosis isolates from patients in informal urban settlements in Nairobi, Kenya. *BMC infectious diseases*, 16, 1-7.
- Lobie, T. A., Woldeamanuel, Y., Asrat, D., Beyene, D., Bjørås, M., & Aseffa, A. (2020). Genetic diversity and drug resistance pattern of Mycobacterium tuberculosis strains isolated from pulmonary tuberculosis patients in the Benishangul Gumuz region and its surroundings, Northwest Ethiopia. *PloS one*, 15(4), e0231320.
- Malenfant, J. H., & Brewer, T. F. (2021). Rifampicin mono-resistant tuberculosis—a review of an uncommon but growing challenge for global tuberculosis control. *Open forum infectious diseases*,
- Manson, A. L., Cohen, K. A., Abeel, T., Desjardins, C. A., Armstrong, D. T., Barry III, C. E., et al. (2017). Genomic analysis of globally diverse Mycobacterium tuberculosis strains provides insights into the emergence and spread of multidrug resistance. *Nature Genetics*, 49(3), 395-402.
- Menzies, D., Benedetti, A., Paydar, A., Royce, S., Pai, M., Burman, W., et al. (2009). Standardized treatment of active tuberculosis in patients with previous treatment and/or with mono-resistance to isoniazid: a systematic review and meta-analysis. *PLoS Medicine*, 6(9), e1000150.
- Mitike, G., Kebede, D., & Yeneneh, H. (1997). Prevalence of antituberculosis drug resistance in Harar Tuberculosis Centre, Ethiopia. *East African medical journal*, 73(3), 158-161.

- Monde, N., Munyeme, M., Chongwe, G., Wensman, J. J., Zulu, M., Siziya, S., et al. (2023). First and second-line anti-tuberculosis drug-resistance patterns in pulmonary tuberculosis patients in Zambia. *Antibiotics*, 12(1), 166.
- Mutayoba, B. K., Ershova, J., Lyamuya, E., Hoelscher, M., Heinrich, N., Kilale, A. M., et al. (2022). The second national anti-tuberculosis drug resistance survey in Tanzania, 2017–2018. *Tropical Medicine & International Health*, 27(10), 891-901.
- OHB. (2018). Data from the Oromia health bureau, 2018. Addis Ababa Ethiopia
- OHB. (2024). Data from the Oromia health bureau, 2024. Addis Ababa Ethiopia
- Otu, A., Umoh, V., Habib, A., Ameh, S., Lawson, L., & Ansa, V. (2013). Drug resistance among pulmonary tuberculosis patients in Calabar, Nigeria. *Pulmonary medicine*, 2013: p. 235190.
- Prasad, R., Gupta, N., & Banka, A. (2018). Multidrug-resistant tuberculosis/rifampicin-resistant tuberculosis: Principles of management. *Lung India: official organ of Indian chest society*, 35(1), 78.
- Rocha, D. M., Viveiros, M., Saraiva, M., & Osório, N. S. (2021). The neglected contribution of streptomycin to the tuberculosis drug resistance problem. *Genes*, 12(12), 2003.
- Seid, A., Girma, Y., Abebe, A., Dereb, E., Kassa, M., & Berhane, N. (2023). Characteristics of TB/HIV Co-Infection and Patterns of Multi-drug-Resistance Tuberculosis in the Northwest Amhara, Ethiopia. *Infection and Drug Resistance*, 3829-3845.
- Seyoum, B., Demissie, M., Worku, A., Bekele, S., & Aseffa, A. (2014). Prevalence and drug resistance patterns of Mycobacterium tuberculosis among new smear-positive pulmonary tuberculosis patients in eastern Ethiopia. *Tuberculosis research and treatment*, 2014.
- Shah, N. S., Wright, A., Bai, G.-H., Barrera, L., Boulahbal, F., Martín-Casabona, N., et al. (2007). Worldwide emergence of extensively drug-resistant tuberculosis. *Emerging infectious diseases*, 13(3), 380.
- Sitienei, J., Kimenye, K., Wahogo, J., Langat, B., Masini, E., Njuguna, O., et al. (2017). 4th national anti-tuberculosis drug resistance survey in Kenya. *J Health Sci*, 5, 282-291.
- Tilahun, M., Wegayehu, T., Wondale, B., Gebresilase, T. T., Gebreyohannes, T., Tekola, A., Alemu, M., et al. (2023). Phenotypic and genotypic drug susceptibility patterns of Mycobacterium tuberculosis isolates from pulmonary tuberculosis patients in Central and Southern Ethiopia. *PLoS one*, 18(9), e0285063.
- van Klingeren, B., Dessens-Kroon, M., van der Laan, T., Kremer, K., & van Soolingen, D. (2007). Drug susceptibility testing of Mycobacterium tuberculosis complex by use of a high-throughput, reproducible, absolute concentration method. *Journal of Clinical Microbiology*, 45(8), 2662-2668.
- Wedajo, W., Schön, T., Bedru, A., Kiros, T., Hailu, E., Mebrahtu, T., Yamuah, L., Ängeby, K., Werngren, J., & Onyebujoh, P. (2014). A 24-well plate assay for simultaneous testing of first and second-line drugs against Mycobacterium tuberculosis in a high-endemic setting. *BMC research notes*, 7(1), 1-8.
- Welekidan, L. N., Skjerve, E., Dejene, T. A., Gebremichael, M. W., Brynildsrud, O., Agdestein, A., et al. (2020). Characteristics of pulmonary multidrug-resistant tuberculosis patients in Tigray Region, Ethiopia: A cross-sectional study. *PLoS one*, 15(8), e0236362.
- WHO. (2009). World Health Organization, Guidelines for Surveillance of Drug Resistance in Tuberculosis, WHO/HTM/TB/2009.422, WHO, Geneva, Switzerland, 4th edition, 2009. <https://www.who.int/publications/i/item/9789241598675>
- WHO. (2013). Definitions and reporting framework for tuberculosis–2013 revision: updated December 2014 and January 2020. In Definitions and reporting framework for tuberculosis–2013 revision: updated December 2014 and January 2020. <https://www.who.int/publications/i/item/9789241505345>
- WHO. (2019). World Health Organization Global tuberculosis report 2019. Geneva: World Health Organization; 2019. <https://www.who.int/publications/i/item/global-tuberculosis-report-2019>
- WHO. (2020a). Global tuberculosis report 2020. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO. <https://www.who.int/publications/i/item/9789240013131>

- WHO. (2020b). Rapid communication: molecular assays as initial tests for the diagnosis of tuberculosis and rifampicin resistance. Geneva: WHO. <https://www.who.int/publications/i/item/9789240000339>
- WHO. (2020c). WHO consolidated guidelines on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment. Geneva: <https://www.who.int/publications/i/item/9789240007048>
- WHO. (2022). Global tuberculosis report 2022. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO. <https://www.who.int/publications/i/item/9789240061729>
- WHO. (2023a). Global tuberculosis report 2023. World Health Organization. <https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023>. 2023.
- WHO. (2023b). World Health Organization WHO consolidated guidelines on tuberculosis. Treatment - drug-resistant tuberculosis treatment, 2022 update. Geneva: World Health Organization; 2022 Global TB report, 2023. <https://www.who.int/news-room/fact-sheets/detail/tuberculosis>.
- WHO. (2024a). Consolidated guidance on tuberculosis data generation and use. Module 1. Tuberculosis surveillance. ISBN 978-92-4-007529-0 (electronic version). World Health Organization (WHO). <https://www.who.int/publications/i/item/9789240075290>
- WHO. (2024b). WHO (2024). Global tuberculosis report 2024. World Health Organization <https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2024>.
- WHO. (2025). WHO consolidated guidelines on tuberculosis. Module 4: treatment and care. Geneva: World Health Organization; 2025. Licence: CC BY-NC-SA 3.0 IGO. <https://www.who.int/publications/i/item/9789240107243>
- Yigzaw WB, T. J., Wang S-H, Tessema B. (2021). Magnitude of phenotypic and MTBDRplus line probe assay first-line anti-tuberculosis drug resistance among tuberculosis patients, northwest Ethiopia. *Infection and Drug Resistance*. 2021:497-505.