

Magnitude of Mycobacterium Tuberculosis and its Rifampicin Resistance using Gene Xpert MTB/RIF Assay among Tuberculosis Suspected Patients at Addis Zemen Hospital, Northwest, Ethiopia: Retrospective Study

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ABSTRACT

Mycobacterium tuberculosis and drug-resistant tuberculosis are alarmingly increasing infectious diseases worldwide. Tuberculosis is the major cause of morbidity and mortality, especially in developing countries like Ethiopia. However, there is scarcity of data on the magnitude of Mycobacterium tuberculosis and rifampicin resistance using the Xpert-MTB/RIF assay in the study area. Therefore, this study aimed to assess the magnitude of Mycobacterium tuberculosis and rifampicin resistance among TB suspected patients using the GeneXpert assay at Addis Zemen Hospital, North West Ethiopia.

Keywords: Gene Xpert; Magnitude; Tuberculosis; rifampicin resistance; Addis Zemen; Ethiopia

INTRODUCTION

Tuberculosis (TB) is a chronic contagious disease primarily caused by the bacillus Mycobacterium tuberculosis (MTB) [1,2]. Although the disease primarily affects the lungs (pulmonary TB), it may also affect other sites (extra-pulmonary TB). Approximately 85% of the estimated number of TB cases are pulmonary [3]. Not everyone infected with TB bacteria becomes sick. As a result, two TB-related conditions exist; latent TB infection and TB disease. If not treated properly, TB disease can be fatal [4].

Despite comprehensive and effective control measures to prevent and control TB disease, it remains a serious public health concern. TB is currently the ninth leading cause of death worldwide, and it was the leading cause of a single infectious agent until the coronavirus (COVID-19) pandemic, ranking above human immunodeficiency virus (HIV). Globally, it kills nearly three people every minute [5,6]. It spreads through the inhalation of bacilli-containing droplets released into the air by untreated people during coughing, sneezing, spitting, or singing [1,2,7]. Furthermore, TB is one of the top ten causes of mortality and the first killer among infectious diseases worldwide. Moreover, multidrug-resistant MTB (MDR-TB), defined as

resistant to at least Isoniazid and rifampicin, has also become a serious global health problem [8].

According to the World Health Organization (WHO) 2021 TB global report, an estimated 9.9 million people have been infected with TB in 2020. In the same year, there were 1.3 million deaths among HIV-negative people and 214,000 deaths among HIV-positive people due to TB. The number of TB deaths increased in 2020 in most of the 30 high TB burden countries. In addition, most TB cases were in South-East Asia (43%), Africa (25%), and the Western Pacific (18%), with smaller shares in the Eastern Mediterranean (8.3%), the Americas (3.0%), and Europe (2.3%). As a result, the 30 countries with a high TB burden accounted for 86% of all estimated incident cases worldwide. Of these, India (26%), China (8.5%), Indonesia (8.4%), the Philippines (6.0%), Pakistan (5.8%), Nigeria (4.6%), Bangladesh (3.6%), and South Africa (3.3%) accounted for two-thirds of the global TB burden [9].

The diagnostic methods accessible for detecting MTB are limited and are frequently less sensitive and poorly suited to low-resource situations [10]. Furthermore, the development of MDR-TB is mainly associated with ineffective TB control programs

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due to inadequate therapy, poor patient compliance, interrupted drug supply, and inappropriate treatment regimens [11]. MDR-TB necessitates an extensive and costly treatment regimen involving toxic and ineffective second-line drugs. Drug resistance is primarily a human-made problem caused by improper drug use and management or a combination of the two [1].

By the year 2035, WHO aims to reduce the incidence and death rate of TB by 90% and 95%, respectively, with the ultimate goal of the total eradication of TB worldwide [12]. Ethiopia is one of the thirty countries with the highest rates of TB, HIV, and MDR-TB, with an estimated yearly TB incidence of 164 per 100,000 population and a death rate of 28 per 100,000 population. In addition, TB case detection in Ethiopia is below the WHO target [13].

Delayed diagnosis of TB is the main contributor to ongoing transmission and failure to achieve successful TB treatment outcomes. Thus, improving early case detection and reporting the actual prevalence is essential for estimating the magnitude, setting the intervention measures, and prioritizing the treatment options in the global fight against TB [6]. The most efficient approach to avoiding the spread of TB in the community is to identify and investigate TB-infected people early in their infection time [14].

The increased prevalence of MDR-TB and extensively drug-resistant TB (XDR-TB) in Ethiopia highlights the critical need for a rapid diagnostic method for TB [6]. Fast and accurate diagnosis of TB and early initiation of appropriate treatment reduces its transmission, magnitude, and mortality. As a result, the Gene-Xpert (Cepheid Xpert® MTB/RIF) assay was approved by the WHO as a first-line tool for TB diagnosis in 2010. This assay is a nucleic acid amplification test that simultaneously detects MTB and rifampicin resistance, widely regarded as a surrogate marker for more than 90% of MDR-TB cases. The assay provides results directly from the clinical specimen with a shorter diagnostic turnaround time (less than 2 hours) than compared with TB culture [6, 15], and the assay is important to detect MTB at a low number [16]. Isolates with mutations in an 81-base-pair region of the RPOB gene, which encodes the ribonucleic acid (RNA) polymerase-subunit, are detected by Gene-Xpert (Cepheid Xpert® MTB/RIF). This locus is an excellent target for rifampin resistance molecular tests [17].

The Gene-Xpert (Cepheid Xpert® MTB/RIF) assay has been used in Ethiopia for the diagnosis of TB at referral and general hospitals since 2014 for HIV and pediatric patients [8]. However, it is now going to be implemented in various health facilities. At Adiss Zemen Hospital (AZH), the diagnosis of TB using the Gene Xpert (Cepheid Xpert® MTB/RIF) test started in May 2018. Data on the local prevalence of MTB and rifampicin resistance-MTB are useful for the prevention and control of MTB, but the magnitude of TB and its resistance to rifampicin using the Gene Xpert assay has not been addressed in the study area. Therefore, this study aimed to assess the magnitude of MTB and its rifampicin resistance using the Gene Xpert-MTB/RIF (Cepheid Xpert® MTB/RIF) assay at AZH, Northwest Ethiopia.

MATERIALS AND METHODS

Study design, area, and period

A hospital-based retrospective cross-sectional study was conducted at AZH from May 2018 to February 2022. The hospital is located in the Amhara Regional State, South Gondar Zone, Libo Kemkem district. It's found to the northwest of Bahir Dar, the capital city of Amhara Regional State, at a distance of 86 kilometers and 573 kilometers from the capital city of Ethiopia, Addis Ababa, in the northwest direction. Furthermore, the hospital serves as a reference laboratory for the diagnosis of TB for nearby health facilities, and it serves over 200,000 people in the catchment area. A retrospective cross-sectional study was conducted from 1st march to 30th march 2022 among patients tested for mycobacterium tuberculosis using genexpert at addis zemen hospital, northwest ethiopia. the data recorded from may 2018 to february 2022 on the genexpert test results registration book was collected using a data extraction sheet. data were entered into epi data version 4.6 and analyzed using stata version 14.0 software

Inclusion and exclusion criteria

The study includes all suspected and confirmed TB cases tested by Gene Xpert (Cepheid Xpert® MTB/RIF) and recorded in the TB registration book during the study period. The study excluded data that lacked sociodemographic information such as age, gender, year of diagnosis, patient registration group, and TB status results.

DATA COLLECTION AND PROCESSING

Data collection

From March 1st, 2022 to March 30th, 2022, data were collected directly from the Gene Xpert (Cepheid Xpert® MTB/RIF) result registration logbook using a standardized data extraction format. The socio-demographic data (age, gender, and residency), patient registration group, TB status result, and HIV status Were collected by b trained data collectors.

Sample size

A total of 3918 data records were collected retrospectively in Gene Xpert's (Cepheid Xpert® MTB/RIF) TB result registration logbook from May 2018 to February 2022Critical.

Laboratory processing

TB from the suspected patients was diagnosed using the Xpert-MTB/RIF (Cepheid Xpert® MTB/RIF) assay from a single sputum sample. After collecting the sputum, the sputum sample was mixed with sample reagent buffer in a volume ratio of 1:2 (sample: sample reagent buffer). The sample container was then tightly closed, vortexed 15 times, and allowed to stand at room temperature for 10 minutes. After 10 minutes, it was vortexed ten times again and allowed to stand for 5 minutes before transferring more than 2 mL of the processed sample (just above the 2 mL mark on the pipette) to the Gene Xpert-MTB/RIF cartridge. The cartridge containing the specimen was then

loaded into the Gene Xpert machine. Finally, after 2 hours, the results were displayed [8].

Quality control of data

Before the start of data collection, the data collectors were trained and given data extraction tools. The principal investigator checked the collected data daily for completeness, accuracy, and consistency. At the end of each data collection, the primary investigator counted and documented the number of verified and suspected cases before data entry. Finally, a random sample of the collected data were selected and checked for completeness, consistency, and accuracy.

Data processing and analysis

The collected data were entered into Epi-Data version 4.6 before being cleaned and analyzed with STATA version 14.0 software. The findings were summarized using descriptive statistics such as mean and percentage. Bivariate and multivariate logistic regression analyses were used to assess factors associated with MTB and MDR-TB. The odds ratio (OR) was calculated at 95% confidence intervals (CI), and a P-value less than 0.05 was considered statistically significant.

Operational definitions

New cases: Patients who have never been treated for TB before the current diagnosis

Relapse cases: patients who were declared cured or treatment completed by a physician but who reported back to the health service and were found to be positive for MTB.

MDR-TB: resistance to at least the two major anti-TB drugs (isoniazid and rifampicin).

Rifampicin Resistant TB: resistance to rifampicin detected by phenotypic or genotypic methods, with or without resistance to other anti-TB drugs.

Ethical consideration

The study was conducted based on the Declaration of Helsinki.

Variables	Categories	Number	%
Sex	Male	2214	56.51
	Female	1704	43.49
Age (in years)	<15	145	3.7
	15-24	458	11.69
	25-44	1496	38.18
	45-64	1274	32.52
	>65	545	13.91
Residence	Urban	2436	62.17
	Rural	1482	37.83
HIV status	Positive	29	0.74
	Negative	637	16.26

The ethical clearance was obtained from the research and review committee of the College of Health Sciences, Debre Tabor University. Informed consent was not applicable since the study was retrospective, and we used the clinical data obtained from the registration log book. A permission letter was also obtained from AZH to conduct the study. Moreover, the confidentiality of the data was kept securely.

RESULTS

Socio-demographic characteristics of the study participants

In this study, a total of 3918 study participants suspected of having TB, had been given sputum samples and had complete data. Of those, the majority of participants were males, 56.5% (2214/3918). The age of the study participants ranged from 1 to 88 years old, with a mean age of 42.8 years and a standard deviation of 17.4 years. The majority of study participants, 38.2% (1496/3918), were aged from 25-44 years old. Besides, most of the study participants, 62.17% (2436/3918), lived in an urban setting. In addition, the unknown HIV status (not tested) of participants accounted for 83.2% (3258/3918), followed by 16.3% (637/3918) of the HIV negative. Furthermore, the majority of study participants, 99.59% (3902/3918), were newly presumptively diagnosed TB patients. Moreover, most of the study participants, 94.9% (3719/3819), were negative for TB. A total of 3918 Mycobacterium tuberculosis suspected patients' samples were tested using the GeneXpert assay. Of these, the majority, 56.5% (2214) of the participants were males. Additionally, about 38.2% (1496) were in the age group of 25-44 years. Furthermore, 62.17% (2436) of the study participants lived in urban areas. The overall magnitude of Mycobacterium tuberculosis was 5.1% (199). Among those, 80.9% (161) of the study subjects had unknown HIV status, and 95.5% (190) were new Mycobacterium tuberculosis cases. The overall prevalence of rifampicin resistance was 4.5% (9). Among those with rifampicin resistance, 66.7% (6) were females, and 66.7% (6) were urban residents. (Table 1).

	Unknown	3252	83
Patient registration group	New	3896	99.44
	Relapse	22	0.56
TB status	Negative	3719	94.92
	Positive	199	5.08
Rifampicin status	Sensitive	190	95.5
	Resistant	9	4.5

Table 1: Sociodemographic and clinical characteristics of study participants at AZH, Northwest Ethiopia, 2022 (N=3918).

Magnitude of Mycobacterium tuberculosis

The overall magnitude of MTB was 5.1% (199/3918), and all were pulmonary TB patients. Of the MTB-positive patients

who were diagnosed by GeneXpert, the majority, 88.9% (117/199) were males, and 44.2% (88/199) were in the age group of 25–44 years. Furthermore, 63.8% (127/199) were urban residents, 80.9% (161/199) of study participants' HIV status was not determined, and 95.5% (190/199) were MTB new cases (Table-2).

Variables	Categories	Result of Gene Xpert for MTB		
		Positive % (N)	Negative % (N)	Total % (N)
Sex	Male	58.8 (117)	56.4 (2,097)	56.5 (2214)
	Female	41.2 (82)	43.6 (1,622)	43.5 (1704)
Age (in years)	<15	4.0 (8)	3.7 (137)	3.7 (145)
	15–24	24.6 (49)	11.0 (409)	11.7 (458)
	25–44	44.2 (88)	37.9 (1,408)	38.2 (1496)
	45–64	18.6 (37)	33.3 (1,237)	32.5 (1274)
	>65	8.6 (17)	14.1 (528)	13.9 (545)
Residence	Urban	63.8 (127)	62.1 (2309)	62.2 (2 436)
	Rural	36.2 (72)	37.9 (1410)	37.8 (1482)
HIV status	Positive	5.0 (10)	0.5 (19)	0.7 (29)
	Negative	14.1 (28)	16.4 (609)	16.3 (637)
	Unknown	80.9 (161)	83.1 (3,091)	83.0 (3252)
Patient registration group	New	95.5 (190)	99.7 (3,7060)	99.4 (3896)
	Relapse	4.5 (9)	0.3 (13)	0.6 (22)

Table 2: The magnitude of MTB detected by Gene Xpert in study participants at AZH in Northwest Ethiopia in 2022 (N = 199).

Magnitude of rifampicin resistance in MTB

The overall magnitude of rifampicin resistance MTB was 4.5 %

(9/199), with 95.5% (191/199) susceptible to rifampicin. Approximately 66.7% (6/9) of the rifampicin resistance patients were females, 66.7% (6/9) were urban residents, 77.2% (7/9) had unknown HIV status, and 88.9% (8/9) were newly diagnosed presumptive rifampicin resistance MTB patients (Table 3).

Variables	Categories	Rifampicin resistance profile		Total % (N)
		Resistance % (N)	Sensitive % (N)	
Sex	Male	33.3 (3)	60.0 (114)	58.8 (117)
	Female	66.7 (6)	40.0 (76)	41.2 (82)
Age	<15	0	4.2 (8)	4.0 (8)
	15–24	33.3 (3)	24.2 (46)	24.6 (49)

	25-44	33.3 (3)	44.7 (85)	44.2 (88)
	45-64	22.2 (2)	18.4 (35)	18.6 (37)
	>65	11.2 (1)	8.4 (16)	8.6 (17)
Residence	Urban	66.7 (6)	63.7 (121)	63.8 (127)
	Rural	33.3 (3)	36.3 (69)	36.2 (72)
HIV status	Positive	0 (0)	5.3 (10)	5.0 (10)
	Negative	22.2 (2)	13.7 (26)	14.1 (28)
	Unknown	77.8 (7)	81.0 (154)	80.9 (161)
Patients' registration group	New	88.8 (8)	95.8 (182)	95.5 (190)
	Relapse	11.2 (1)	4.2 (8)	4.5 (9)

Table 3: Rifampicin resistance severity in MTB positive study participants at AZH, Northwest Ethiopia, 2022 (N = 9).

DISCUSSION

Drug resistance to MTB is rapidly increasing and occurring across the globe, specifically in developing countries, posing a severe threat to public health [18]. Similarly, diagnosis of MTB in developing countries largely depends on smear microscopy that has poor sensitivity, which results in an increased smear-negative pulmonary TB and remains undiagnosed or delayed diagnosis and treatment [19].

In the current study, the overall magnitude of MTB was 5.1% (95% CI, 4.4%-5.8%). This finding is lower than the previous study results in Debre Tabor and Felege Hiwot hospital, Ethiopia, 14.6% (6); Motta, Ethiopia, 8.4% (1); Debre Markos, Ethiopia, 23.2% (20); Haramaya, East Ethiopia, 7.8% (14); Aksum, Northern Ethiopia, 7.9% (8); Lagos, Nigeria, 37.7% [21], Nepal, 9.9% and 13.8% [22, 23]. Variation in the study population, setting and locations where the study was conducted, sample size, climatic conditions, diagnostic methodology, and type of study design used could all be reasons for such a disparity.

In this study, the magnitude of rifampicin resistance MTB was 4.5% (95% CI, 2.3-8.5%). The finding of the present study is in line with that of the previous studies conducted in Dessie and D/Birhan, Northeast Ethiopia, 2.6% (2), Dubti Hospital, Afar, Ethiopia, 4.3% [24], East Gojjam, Ethiopia, 3.89% [25], Motta, Ethiopia, 4.3% (1), Ataye, Ethiopia, 5.3% [26], Tigray, Northern Ethiopia, 3.5% [27], Southeast Ethiopia, 4.35% [28], and Gedeo Zone, Southern Ethiopia, 5.1% (18), and Botswana, 5.4% [29]. However, in the present study, the magnitude of rifampicin resistance MTB is lower than the study results done in Debre Tabor and Felege Hiwot hospitals, Ethiopia, 9.3% (6), University of Gondar Hospital, Ethiopia, 15.8% (17), Debre Markos Referral Hospital, Ethiopia, 10.3% (20), Tigray, Northern Ethiopia, 9% (8), Adigrat General Hospital, North Ethiopia, 9.1% (4), Makkah, Saudi Arabia, 17.1% [30], and Lagos, Nigeria, 23.4% (21). But the magnitude of rifampicin resistance MTB in the current study is higher than in a study done in a district hospital in India, 2.2% (31). These differences might be explained by geographical variation, methodological differences, sample size, differences in the study period,

differences in TB control and prevention practice, and the scope of using the Gene Xpert assay for TB diagnosis.

In this study, the proportion of rifampicin-resistant MTB was higher among treatment-naive patients than in previously treated patients. This might be due to the active transmission of the bacteria or the presence of new undiagnosed rifampicin-resistant TB cases. Moreover, drug resistance among previously untreated cases showed that the performance of TB control programs in the past was poor.

In this study, the proportion of males positive for MTB was higher than that of females. This finding is supported by other studies [20,23]. This difference between males and females could be attributed to differences in social and health-seeking behavior, environmental factors, and males' tendency to be more exposed to the outside environment; smoking and alcoholism are factors that increase the risk of contracting MTB bacilli [23]. Furthermore, a high proportion of young adults were infected with MTB. This finding is supported by a previous study [4]. In addition, urban residents have a higher proportion of being positive for MTB and MDR TB. This could be due to the crowded living conditions of people in urban areas, which facilitate the transmission of the disease.

CONCLUSION

Even though there is a scale-up of intervention and prevention measures in the country, this study showed that TB together with rifampicin resistance is still the major public health problem in the study area. Males are more infected than females. Higher rifampicin resistance was found among females and urban residents. Therefore, strengthening the existing intervention and prevention measures has to be a great concern of the stakeholders to reduce the magnitude of TB and rifampicin resistance.

LIMITATION OF THE STUDY

We could not assess many variables since the data were gathered retrospectively from the GeneXpert TB registration logbook. In addition, the various variants of MTB were not separated because the Gene Xpert assay cannot determine the strain of MTB.

DECLARATION

Ethics approval and consent to participate

The study was conducted based on the Declaration of Helsinki. The ethical clearance was obtained from the research and review committee of the department of Medical Laboratory Sciences, College of Health Sciences, Debre Tabor University. Informed consent was not applicable since the study was retrospective, and we used the clinical data obtained from the registration log book. A permission letter was also obtained from AZH to conduct the study. Moreover, the confidentiality of the data was kept securely.

Consent for publication

Not applicable

Availability of data and materials

All data supporting the findings of this study are available within the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest.

FUNDING

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AUTHORS' CONTRIBUTIONS

AB: Conception; acquisition, analysis; interpretation of data; drafted the work and revised the manuscript, **BS:** acquisition, analysis, and interpretation of data, **BG:** revised the manuscript, **SW:** acquisition, analysis, and interpretation of data, **GA:** acquisition, analyzed, and revised the manuscript, **BeM:** Collection, acquisition, analysis, and interpretation of data, **SDa:** acquisition, analyzed, and revised the manuscript, **SDi:** interpretation of data and revised the manuscript, **TD:** interpretation of data and revised the manuscript, **BiM:** analysis, interpretation of data and revised the manuscript, **DT:** interpretation of data and revised the manuscript, **TK:** Methodology, interpretation of data and revised the manuscript, **AA:** Conception, methodology, original draft, analysis, and interpretation of data; and revised the manuscript.

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ABBREVIATIONS

AZH: Addis Zemen hospital; CI: confidence intervals; MDR-TB: multidrug resistance Tuberculosis; MTB: Mycobacterium tuberculosis; RIF: rifampicin; HIV: human immunodeficiency virus; TB: Tuberculosis; WHO: world health organization

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