

Xpert MTB/RIF assay for the diagnosis of *Mycobacterium tuberculosis* and Rifampicin resistance in high Human Immunodeficiency Virus setting in Gambella regional state, southwest Ethiopia

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ABSTRACT

Background: The introduction of a new and rapid molecular diagnostic for tuberculosis (TB) and rifampicin resistance (RIF) in the national TB program has improved the diagnosis of TB by shortening the turnaround time and detecting the presence of RIF resistance in high TB and human immunodeficiency virus (HIV) settings such as Ethiopia. However, the implementation of this new diagnostic tool for the diagnosis of *M. tuberculosis* (MTB) and rifampicin (RIF) resistance in clinical setting is limited known in the country. Hence, this study intended to describe the program of GeneXpert MTB/RIF in the diagnosis of TB and RIF in high HIV setting in Gambella Regional State, Southwest Ethiopia.

Method: Institutional based retrospective study was conducted among presumptive TB patients diagnosed with GeneXpert assay in the last three years (2015–2017) in Gambella Hospital from May 1–30, 2017. The data were collected from GeneXpert registration book using standard data extraction sheet. The data were entered and cleared using EPI data 3.1 and then, exported and analyzed using SPSS version 20.0 statistical software package.

Result: Of the 995 presumptive TB patients who received the GeneXpert test in the last three years, 20.0% (95% CI: 17.4–22.7) of them had proven MTB detection while 4.9% (95% CI: 2.2–8.1) had RIF resistance. The prevalence of RIF resistance was 2.3% and 14.3% among the new and retreated cases respectively. There was also a 35.5% TB/HIV co-infections. The odd of MTB detection was higher among 15–29 [AOR 2.17 (95% CI: 1.25–3.76)] and 30–44 [AOR 2.35 (95% CI: 1.36–4.07)] year old participants. The figure was however significantly lower among female [AOR 0.64 (95% CI: 0.45–0.91)] and unknown HIV status [AOR 0.38(95% CI: 0.24–0.61)] participants of the study. In addition, the odd of RIF resistance was significantly low among HIV unknown case [AOR 0.14 (95% CI: 0.02–0.96)]. It was also learnt that there was progressively decline of invalid or error Xpert result from 4.7% to 2.0% in the course of the study period (X^2 , 25.54; $P = 0.001$).

Conclusion: The study confirms the high prevalence of TB, RIF resistance and TB/HIV co-infection among the study participants. Age, sex, and HIV status of the study participants were predictor factors for MTB detection while HIV status was associated with RIF resistance. Therefore, the results of the study indicate that there is the need for collaborative and intensified prevention of TB and HIV in the study area. The ongoing supervision and mentoring to improve the performance of Xpert in the institution need to be promoted.

Introduction

Today, TB remains one of the leading causes of death globally even though most TB cases could be cured on the bases of timely diagnosis and proper treatments. In 2015, 10.4 million new TB cases and 1.4 million deaths occurred worldwide. Developing countries in the sub-Saharan Africa bear the greater proportion of TB burdens [1]. Ethiopia is one of the top 10 high TB burden countries in the world [1]. The disease

is the major cause for morbidity and mortality in the country [2]. The country is also among the list of high burden drug resistance TB and TB/HIV co-infected countries [1].

TB care and control gives priorities in improving case-detection and diagnostic capacity of multidrug-resistant tuberculosis (MDR-TB). However, the existing conventional diagnostic techniques in routine clinical setting misdiagnosed the HIV co-infected TB and young age cases [3]. Accordingly, WHO recommended a novel and rapid

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diagnostic Xpert MTB/RIF assay for diagnosis of pulmonary TB among adults in 2010. This helps to make a simultaneous detection of MTB and resistance to RIF, a surrogate marker for MDR-TB. The Xpert detects *rpoB* mutation that was found in 96.1% of RIF-resistant MTB strains worldwide. Since 2013, WHO has also recommended the Xpert for extra-pulmonary specific form TB and children [4].

Sputum smear microscopy remains the common diagnostic method for TB in clinical setting in Ethiopia despite the method has low sensitivity [5]. The golden standard for TB diagnosis AFB culture is unavailable and usually requires long period to obtain the result [6,7]; and it is found only in regional and national referral hospitals and research centers in the country. As Ethiopia is one of the 21 recipient countries to implement the TB Xpert project, the country has started implementations since 2014. National guideline recommends Xpert MTB/RIF to be used for presumptive MDR-TB cases and presumptive TB cases among HIV positive individuals, and children below 14 years of age [2]. Previous studies in Ethiopia have assessed the importance of the introduction of GeneXpert MTB/RIF program in the country [8-16]. However, there is limited evidence on the program implementation of Xpert MTB/RIF for the diagnosis of MTB and RIF resistance in clinical setting in Ethiopia [18]. Hence, this study aims to describe the Xpert MTB/RIF assay program for the diagnosis of MTB and RIF resistance in high HIV setting in Gambella Regional State, Southwest Ethiopia.

Methodology

Study setting, design and period

The study was conducted in Gambella Hospital located in Gambella Town. The hospital belongs to the government of Gambella Regional State. It provides services for the entire population of the state. Majority of peoples of the region are pastoral communities. The state has large landmass border with South Sudan, and faces regular instability among ethnic groups living in the region. The region hosts significant number of refugee in the country as result of proximity to the South Sudan. The Xpert has been introduced to the region since November 2014. The Gambella Hospital provides referral and diagnostic services of Xpert test for the regional communities and refugees living in the state. The region is one of the high HIV prevalent regional states in the country. This retrospective study was conducted to evaluate Xpert program implementation in this pastoral and high HIV affected region from May 1–30, 2017.

Data collection procedure

The data were retrieved using pre-prepared data extraction sheet from GeneXpert registration log book. Patient's sex, age, patient category, HIV status and Xpert results were extracted for each presumptive TB patient diagnosed using Xpert during the course of the study year (November 2014 to March 2017). And patients who lack the Xpert result were excluded from the analysis. The data were collected by trained laboratory personnel working at GeneXpert unit of the institution.

The presumptive TB patients were categorized into four groups based on their history of previous treatment: **New cases:** presumptive TB cases those who have been never treated for TB; **Relapse cases:** those who have been previously treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now presents with a recurrent episode of symptoms or signs suggestive of TB; **Failure cases:** those who have been previously treated for TB and whose treatment failed at the end of their most recent course of treatment, and now come for Xpert diagnosis; and **Defaulter cases:** presumptive TB cases who have been previously treated for TB and were declared loss to follow-up at the end of their most recent course of treatment because of interruption for two or more consecutive months for any reason without medical approval.

Xpert MTB/RIF assay

The Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA) was performed according to the manufacturer's instructions and national guideline [2,19]. As per the protocol, the collected morning sputum sample (3–5 ml) was treated with sample reagent containing NaOH (5–10%) and isopropyl alcohol (10–20%) with a ratio of two sample reagent (2 ml) to one sputum sample (1 ml). The mixture was vortex homogenized, incubated for 15 min at room, and then, transferred into the Xpert MTB/RIF cartridge, and loaded into the GeneXpert machine that process the samples automatically. The primer in Xpert assay amplified conserved region of *rpoB* gene for MTB isolate. The probes are able to identify presence of mutations in the core region that are associated with RIF resistance. The assay was also used to determine the load of MTB using the manufacturer's software based on the threshold cycle values of MTB positive samples.

The internal quality control of Xpert machine was validated using *M. tuberculosis* H37Rv (non RIF resistant) and known RIF resistant isolates stored in –20 °C this hospital. In addition, Xpert machine has inbuilt quality monitoring system. These are Probe Check (PC): to control any system problem related to probe and Sample Process Control (SPC): to monitor any error occurs during sample processes. Moreover, to improve overall Xpert performance of Gambella Hospital, the hospital was periodically monitored by the Ethiopia National TB Reference Laboratory (ENTBRL). The ENTBRL will send them blind Panel Tests (External Quality assurance) of known result quarterly. Then Gambella Hospital will process and resend back panel test result to ENTBRL. The ENTBRL will give feedback and training based on the result.

HIV testing

HIV test was done by a tiebreaker regimen consisting of three rapid HIV (1 + 2) tests in the hospital following the national algorithm recommend by the Federal Ministry of Health [20]. HIV (1 + 2) Antibody Colloidal Gold (KHB, Shanghai Kehua Bio-engineering Co Ltd, China) test is run first on whole blood, and any positive samples are then confirmed using the second test (HIV 1/2 STAT-PAK (Chembio Diagnostics, USA). Any discordant results were resolved using a third confirmatory testing kit, HIV-1/2 Unigold Recombinant assay (Trinity Biotech, Ireland). Invalid tests where the control line did not appear were discarded and repeated on new test devices according to manufacturers' instructions.

The HIV test was processed following manufacturers' instructions and Institutional Standard Operation Procedure. The quality of each kits used for HIV test was validated using known HIV positive and negative stored serum in the center. The performance of the diagnostic center was monitored by the Gambella Regional Laboratory and Ethiopia National HIV/AIDS Reference Laboratory though on site periodical supervision and sending blind Panel Test with known result.

Variables

Xpert results were the dependent variable of the study while age, sex, HIV infection, Patient category and diagnosis years were the independent variables.

Data management and analysis

The collected data were double entered using EPI data 3.1 software; checked for inconsistencies and cleaned accordingly. We used SPSS software version 20.0 for the statistical analysis after importing the EPI data. Descriptive statistics such as relative frequencies and proportion were used to describe qualitative variables and mean and standard deviation (SD) for continuous variables. A final model, multivariate logistic regression was computed by including factors found to be

Table 1
Patient profile of presumptive drug resistance TB patients in Gambella Hospital, 2015–2017.

Variable	Frequency	Percentage	
Age	1–14	180	18.1
	15–29	280	28.1
	30–44	279	28.0
	45–64	150	15.1
	>65	35	3.5
	unrecorded	71	7.1
Sex	Male	553	55.6
	Female	414	41.6
	Unrecorded	28	2.8
Patient category	New	481	48.3
	Relapse	123	12.4
	Defaulter	7	0.7
	Failure	18	1.8
	Unrecorded	366	36.8
HIV status	Positive	96	9.6
	Negative	141	14.2
	Unknown	758	76.2
Year	Nov, 2014- Aug, 2015	233	23.4
	Sept, 2015-Aug, 2016	258	25.9
	Sept,2016-Mar, 2017	504	50.7

Note: Nov, November; Sept, September; Aug, August; Mar, March.

significant at P-value < 0.05 in Chi-square test to identify independent predictors for MTB detection and RIF-resistance. Statistical significance was evaluated at 95% levels of significance. Those variables with p-value < 0.05 on the final model were identified as the associated risk factors for MTB detection and RIF-resistance.

Ethical consideration

The study was conducted in accordance with the ethical clearance obtained from Jimma University College of Health Sciences, Instructional Research and Ethical Review Board (HRPGC/40192/2016). Official permission was also obtained from the regional health department and hospital administration. The confidentiality of information related to study participants was assured during and after the data collection.

Result

As summarized in Table 1 and Fig. 1, a total of 995 TB suspected patients eligible for GeneXpert assay were included in this study. Majority (56.1%) of study participants were between 15–44 years of age. The mean age of the study participants was 30.1 with standard deviation of 17.14 (Range from 1 to 90 years). The male participants (55.6%) account for the large proportion of presumptive TB cases. Among the patients with known HIV sero-status, 40.5% (96/237) were reactive for HIV. With regard to patient category, new patients take the larger proportion followed by defaulter and failure in previous TB treatments.

Out of the total presumptive TB patient analyzed using Xpert assay in the last three years, the prevalence of MTB was 20.0% (95% CI: 17.4–22.7) for those with valid result. Of these, 176(17.7%), 9(0.9%) and 8(0.8%) were susceptible, RIF resistance and RIF indeterminate, respectively. There was also low (3%) level of invalid or error result (Fig. 1 and Fig. 2).

It was also learnt that there was irregularity in the trends of MTB and RIF resistance detection in the last three years. However, progressively decline of invalid or error Xpert result was observed from 4.7% to 2.0% in the course of the study period (X^2 , 25.54; $P = 0.001$) (Table 2).

The prevalence of MTB among the study participant was found to be: male (22.2%), between the age of 15–29 (22.6%) and 30–44 (25.0%) years old, relapse (28.8%), defaulter (42.9%), failure (47.1%)

and, HIV positive (35.5%). Multivariable logistic regression revealed that after adjusting for other variables, MTB was significantly higher among 15–29 [AOR 2.17 (95% CI: 1.25–3.76)] and 30–44 [AOR 2.35 (95% CI: 1.36–4.07)] years old compared to children age group. On the other hand, MTB prevalence was significantly lower among female [AOR 0.64 (95% CI: 0.45–0.91)] and unknown HIV status [AOR 0.38(95% CI: 0.24–0.61)] participants of the study (Tables 3).

We have excluded participants with indeterminate RIF resistance and invalid/error Xpert result in RIF resistance prevalence analysis. The prevalence of RIF resistance was 4.9% (95% CI: 2.2–8.1) among presumptive TB patients while that of RIF resistance was 2.3% and 14.3% among new and retreated case, respectively. RIF resistance was significantly lower for HIV unknown [AOR = 0.14(95%CI: 0.02–0.96)] study participants (Table 4).

Discussion

Monitoring and evaluation of the GeneXpert program in the course of implementation do have immense contribution to provide on time corrective measures in addition to its contribution in indicators requisition need in expending the program in other health care system. With this essence, this study was conducted to evaluate the program implementation of Xpert MTB/RIF assay for diagnosis of MTB and RIF resistance in high HIV setting in Gambella Regional State, Southwest Ethiopia. As a result, out of 995 TB presumptive patients who were eligible for GeneXpert assay, 20.0% had proven MTB detection in this study. This result is consistent with the findings of previous studies carried out in Debre Markos Referral Hospital (23.2%) [17], Gambo Hospital (20.0%) [10], Bahirdar (24.3 %) [21], South Asian General Hospital (21.32%) [22], Northern Nigeria (23%) [23], India (27.6%) [24], and South Africa (26%) [25]. However, it was lower compared to other reports in Nigeria (31.4%) [26]. The observed difference may be attributed to study population differences, where our study participants were presumptive TB patient eligible for Xpert assay because of their HIV sero-staus, age, retreated and/or contact with known MDR-TB patient as per national guideline [2]. On the contrary, this prevalence is higher than previous studies carried out in Southwest Ethiopia [9], North Ethiopia [18], Addis Ababa [27], Seka Health Center [28] and Korea [29]. The difference may be partially related to the method used to detect the TB. The study in Addis Ababa and Seka Health Center for instance used direct microscopy to determine the prevalence of TB among all TB suspected cases. In addition, geographical and study population differences among the studies may have contributed for the observed differences.

In this study, a low invalid result and a progressive improvement was also observed in the course of the study period in the implementation of Xpert. This is attributed to the contribution of continuous supervision and monitoring from the National TB Control Program. Moreover, the design of Xpert (real time PCR) by itself contributed for the occurrence of limited analytical errors in the course of sample process [19].

In a multivariate regression model, this study showed a significant association between age group of 15–44 years old and the risk of contracting MTB infection. This observed presence of higher TB among this productive age groups is in agreement with pervious report from UK [30], global WHO report [1] and studies in Ethiopia [9,10,17,18]. This is a good indicator for the impact of TB on the national productive age group of the country. This higher burden TB among this productive age group is likely due to high mobility from place to place, increased risky sexual behaviors and environmental exposure that hastens the acquisition of TB infection as compared to other age groups.

In the present study, there were 0.64 lower odd of MTB detection among the female. This is in agreement with the global burden of TB [1] and other report in Ethiopia [17]. It is attributed to lower exposure of female to the outer environment, mobility from place to place and TB risk activities like smoking and alcoholism [31]. Interestingly, this

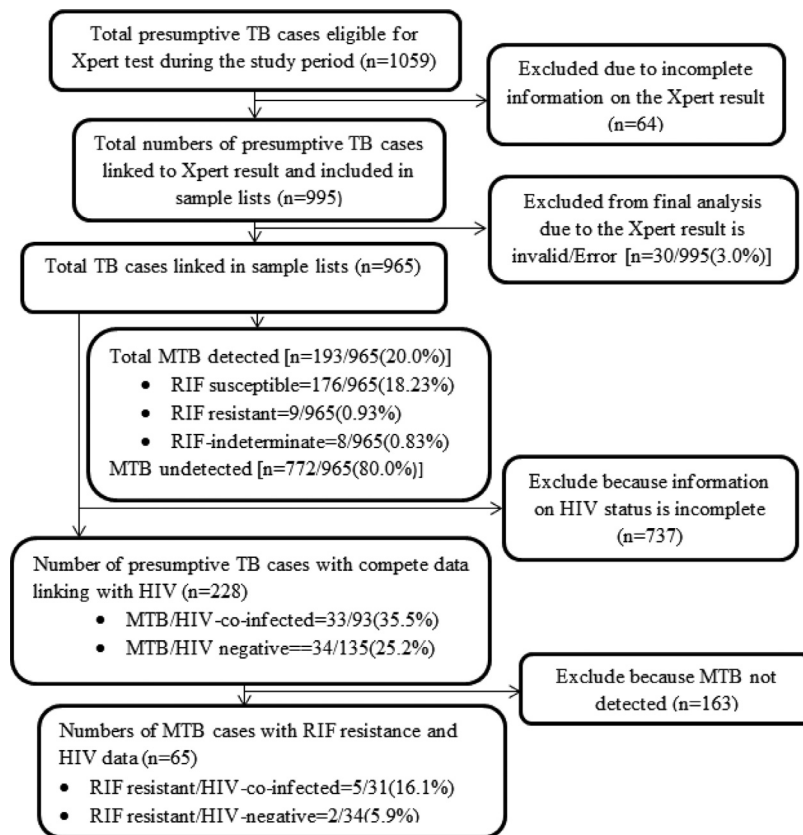


Fig. 1. Numbers of presumptive TB cases screened and analyzed with their Xpert result outcome, MTB/HIV-co-infection, RIF resistance/HIV-co-infection in Gambella Hospital, 2015–2017. Note: TB, tuberculosis; HIV, human immunodeficiency virus; RIF, rifampicin; MTB, M. tuberculosis.

study shows a significant correlation between HIV and MTB detection. The observation supports the existing literature elsewhere [1,17,32].

The prevalence of TB/HIV co-infection rate was found to be 35.5%. The finding is however higher than studies conducted in Debre Markos Referral Hospital (10%) [8], Nigeria (12.5%) [33], and WHO estimation on TB/HIV co-infection in Ethiopia 19.1% [1]. It was also lower

(41.9%) than the findings of the study conducted in Northwest Ethiopia [18]. The possible reason for such discrepancy could be the prevalence of HIV variation in different communities and the level of HIV testing performed. In addition, use of HIV infection as one of the eligible criteria for Xpert test for TB diagnosis could contribute also [2].

In this study, RIF resistance, which is a surrogate marker for MDR-

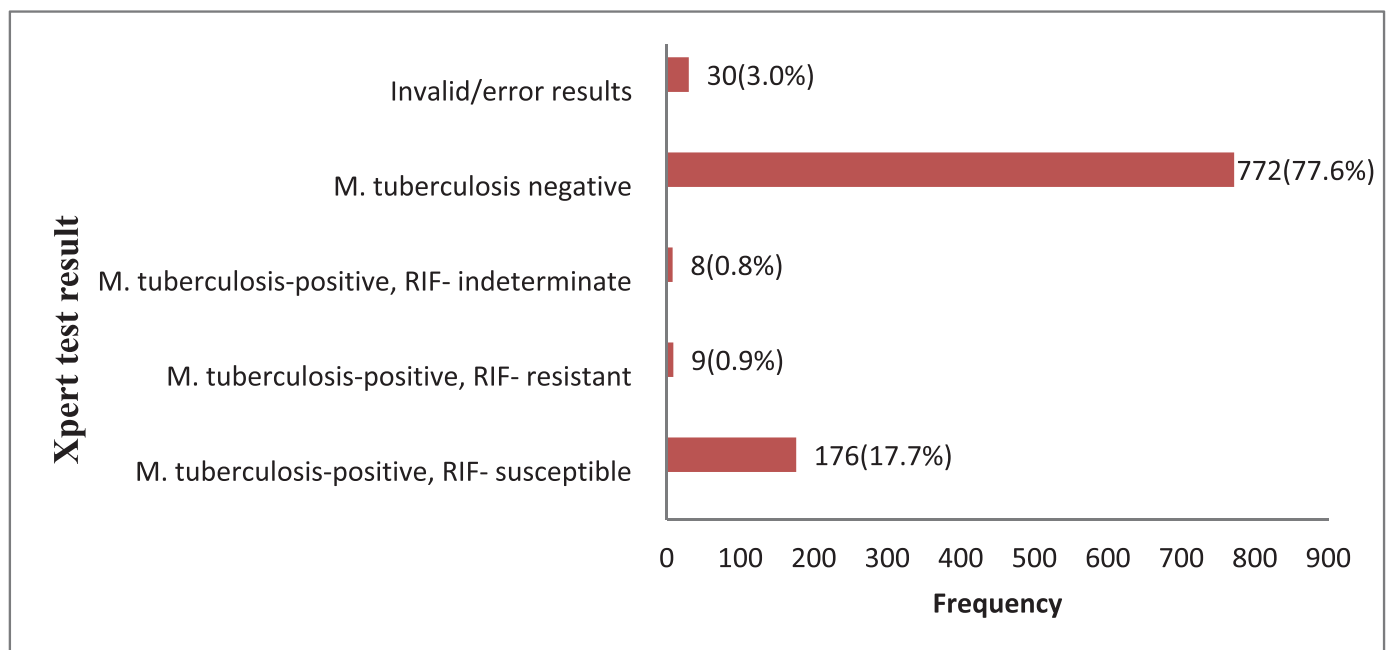


Fig. 2. Xpert test result of presumptive drug resistance TB patients in Gambella Hospital, 2015–2017.

Table 2
Trend of Xpert result in the last three years in Gambella Hospital, 2015–2017.

Year	Xpert result, n (%)						χ^2 -value	P-Value
	M.TB positive-RIF Susceptible	MTB Positive RIF resistant	MTB positive RIF-Indeterminate	MTB Negative	Invalid/Error			
Nov, 2014- Aug, 2015	37(15.9)	0(0.0)	1(0.4)	184(79.0)	11(4.7)	25.54	0.001*	
Sept, 2015-Aug, 2016	58(22.5)	6(2.3)	5(1.9)	180(69.8)	9(3.5)			
Sept,2016-Mar, 2017	81(16.1)	3(0.6)	2(0.4)	408(81.0)	10(2.0)			
Total	176(17.7)	9(0.9)	8(0.8)	772(77.6)	30(3.0)			

Note: RIF, Rifampicin; MTB, *M. Tuberculosis*; *, significant, Nov, November; Sept, September; Aug, August; Mar, March.

Table 3
Prevalence and associated factors of *M. Tuberculosis* using GeneXpert among study participants in Gambella Hospital, 2015–2017.

Characters		<i>M. tuberculosis</i>		Total n (%)	χ^2 - value	P-value	AOR(95%CI)	P-value	
		Detected N (%)	Undetected N (%)						
Age	1–14	20(11.5)	154(88.5)	174(18.0)	16.92	0.005*	1.00	0.006*	
	15–29	62(22.6)	212(77.4)	274(28.4)			2.17(1.25- 3.76)		
	30–44	68(25.0)	204(75.0)	272(28.2)			2.35(1.36–4.07)		0.002*
	45–64	21(14.5)	124(85.5)	145(15.0)			1.24(0.64–2.40)		0.529
	> 65	6(17.6)	28(82.4)	34(3.5)			1.63(0.59–4.47)		0.345
	unrecorded	16(24.2)	50(75.8)	66(6.8)			1.67(0.71–3.95)		0.243
Sex	Male	120(22.2)	421(77.8)	541(56.1)	13.63	0.001*	1.00	0.013*	
	Female	62(15.6)	335(84.4)	397(41.1)			0.64(0.45–0.91)		
	Unrecorded	11(40.7)	16(59.3)	27(2.8)			2.21(0.83–5.90)		0.112
Patient category	New	91(19.6)	374(80.4)	465(48.2)	19.57	0.001*	1.00	0.278	
	Relapse	34(28.8)	84(71.2)	118(12.2)			1.32(0.80–2.17)		
	Defaulter	3(42.9)	4(57.1)	7(0.7)			2.68(0.52–14.02)		0.241
	Failure	8(47.1)	9(52.9)	17(1.8)			2.75(0.97–7.76)		0.057
	Unrecorded	57(15.9)	301(84.1)	358(37.1)			0.85(0.57–1.25)		0.399
HIV status	Positive	33(35.5)	60(64.5)	93(9.6)	20.08	0.000*	1.00	0.102	
	Negative	34(25.2)	101(74.8)	135(14.0)			0.62(0.34–1.10)		
	Unknown	126(17.1)	611(82.9)	737(76.4)			0.38(0.24–0.61)		0.000*

Note: AOR, Adjusted Odds ratio; CI, Confidence interval; 1.00, Reference; *, significant

Table 4
Prevalence and predictors of RIF resistance among presumptive drug resistance patients in Gambella Hospital, 2015–2017.

Characters		RIF Resistance pattern, n (%)		Total, n (%)	χ^2 - value	P-value	AOR(95%CI)	P-Value
		Resistance	Sensitive					
Age	1–14	0(0.0)	18(100.0)	18(9.7)	8.26	0.142	1.00	0.059
	15–29	1(1.6)	60(98.4)	61(33.0)				
	30–44	6(9.2)	59(90.8)	65(35.1)				
	45–64	0(0.0)	20(100.0)	20(10.8)				
	> 65	0(0.0)	5(100.0)	5(2.7)				
	unrecorded	2(12.5)	14(87.5)	16(8.6)				
Sex	Male	4(3.5)	111(96.5)	115(62.2)	5.43	0.066	1.546(0.11–0.95)	0.743
	Female	3(5.0)	57(95.0)	60(32.4)				
	Unrecorded	2(20.0)	8(80.0)	10(5.4)				
Patient category	New	2(2.3)	86(97.7)	88(47.6)	10.43	0.005*	0.32(0.05–1.87)	0.206
	Retreated	6(14.3)	36(85.7)	42(22.7)				
	Unrecorded	1(1.8)	54(98.2)	55(29.7)				
HIV status	Positive	5(16.1)	26(83.9)	31(16.8)	11.23	0.004*	0.14(0.02–0.96)	0.045*
	Negative	2(5.9)	32(94.1)	34(18.4)				
	Unknown	2(1.7)	118(98.3)	120(64.9)				

Note: AOR, Adjusted Odds ratio; CI, Confidence interval; 1.00, Reference; *, significant.

TB (resistant to at least RIF and isoniazid), was detected among 4.9% (95% CI: 2.2–8.1) of the study participants. The RIF resistance was 2.3% among the new and 14.3% in retreated TB case. This level of RIF drug resistance in our study is comparable to the national TB drug resistance among the new (2.7%) and retreated (17.9%) cases [34], and other studies conducted in Gonder (4.2%) [8], Uganda (3.5%) [35], and Nigeria (6.9%) [33]. It is however less than the studies conducted in Debre Markos Referral Hospital (9.8%) [8] and Northwest Ethiopia (9.3%) [18]. Our finding is still higher than the study conducted on referral sample in Ambo, Oromia Regional State, Ethiopia (0.3%) [10]. This higher RIF resistance in this study also urges for an immediate

phenotypic study in order to use rifampicin resistance as surrogate marker for MDR-TB in the area, according to WHO's recommendation. As per the recommendation, the non MDR-TB rifampicin resistance should be less than 3% as a good quality performance indicator [36].

The presence of negative correlation between HIV unknown status with RIF resistance when comparing with presence of HIV infection was support the need for providing HIV testing for any presumptive drug resistance TB patients. This partially justify also the importance of knowing HIV status in clinical management of TB to prevent the impact of impaired immunity by HIV/AIDS on TB proliferation and treatment response.

This study has been undertaken with its own limitations. First, it is institution-retrospective study where there were a numbers of unrecorded variables in the study. Second, even though it is not objective of this study to evaluated performance of the test (sensitivity, specificity, Positive predicative value and Negative predicative Value), the study lacks comparators such as culture, sputum smear and chest X-ray result as Xpert test result is a sole diagnostic for TB and RIF resistance for eligible cases in the study setting. Despite these limitations, the study provides baseline information on implementation of Xpert for detection of MTB and RIF resistance in high HIV setting in Gambella Regional State, Ethiopia.

Conclusion

The study confirms the higher prevalence of TB, RIF resistance and TB/HIV co-infection among the study participants. Age, sex, and HIV status of the study participants were predictor factors for MTB detection while HIV status was associated with RIF resistance. There was a low and progressive improvement in the invalid Xpert results. Therefore, the results of the study urge the need for collaborative and intensified prevention of TB and HIV infection in study area. The ongoing supervision and mentoring for improving the performance Xpert in the institution also need to be promoted.

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Author contribution

EE, GB, ZB, GA conceived and designed the protocol. EE and ZB performed the data collection and contributed for data analysis, and wrote the paper. All authors read and approved the final paper. All authors contributed equally to this work.

Conflict of interests

We, the authors, declare that we have no conflict of interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jctube.2018.06.002.

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