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Xpert MTB/RIF assay for the diagnosis of Mycobacterium tuberculosis and its Rifampicin resistance at Felege Hiwot and Debre Tabor Hospitals; Northwest Ethiopia: A preliminary implementation research

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Abstract

Background: The World Health Organization in 2010 indorsed Xpert MTB/RIF (Xpert) assay for the diagnosis of tuberculosis and multidrug resistant tuberculosis. However, the use of this novel diagnostic method is still limited in a high TB and human immunodeficiency virus burden settings including Ethiopia. Therefore, we conducted this study to describe the first implementation result of Xpert assay in the diagnosis of TB, TB/HIV and MDR-TB at Felege Hiwot Referral Hospital (FHRH) and Debre Tabor General Hospital (DTGH), Northwest Ethiopia.
Methods: We analyzed the records of 1922 (FHRH=544 and DTGH=1378) presumptive TB patients diagnosed using Xpert test from 2015 to 2016 at FHRH and DTGH, Northwest Ethiopia. Information on the demographic and clinical data was collected. Data were entered, cleared, and analyzed using SPSS statistical software package; $p < 0.05$ was considered to be significant.
Results: Overall Xpert assay properly diagnosed 14.6% of the cases (258/1922). Among this rifampicin (RIF) resistance was detected at 9.3% (24/258) of the cases. In the studied region, clinical data reported that 81.0% (1556/1922) of the cases were MDR- TB. Among the study subjects, 888 (46.2 %) of them were HIV positive. TB-HIV co- infection rate was at 41.9% (108/258). Of the total patients registered, 1005 (52.3%) of whom were males. The mean age of patients was 31.1 years with SD of 17.5. Significant predictors of the Xpert test were: age (p=0.000), sex (p=0.009), HIV (p=0.003) and presumptive MDR-TB (p=0.000).

Conclusions: In the studied areas, large proportion of clinically TB suspected patients were wrongly diagnosed with multidrug resistant TB. Therefore, the use of Xpert assay in health settings with no culture facility will decrease the unnecessary use of anti-TB drugs and improve rapid TB, TB/HIV and MDR-TB detection and proper management of the cases.
Key Words: TB, GeneXpert MTB/RIF assay, FelegeHiwot Referral Hospital, DebreTabore General Hospital, Northwest Ethiopia.

Background

Tuberculosis (TB) is one of the oldest diseases known to affect humans, infects approximately one third of the world's population [1, 2]. The World Health Organization (WHO) in 2015 reported that there were an estimated 9.6 million new TB cases and 1.5 million deaths (WHO, 2015). Regardless of having highly efficacious treatment for decades, TB remains the main public health problem [1, 2].

Africa and Asia, where the highest prevalence of co-infection with HIV is reported accounted the highest proportion of TB worldwide [3, 4]. The WHO report in 2009 indicated that TB is a disease of poverty in which 90% of the disease occurred in the developing countries [5]. Ethiopia is highly affected by the TB pandemic and is ranked seventh among the 22 high-burden TB countries worldwide [6]. Ethiopia is one of the high TB, TB/HIV and MDR-TB countries listed (WHO, 2015).The global priorities for TB care and control are to improve early case-detection and treatment. Delayed diagnosis of TB is a major factor to the continued transmission and failure to the successful TB treatment outcome reported [7]. The emergence of MDR-TB is a significant challenge for TB control and prevention programme [8]. The increased in MDR-TB and extensively drug-resistant TB (XDR-TB) incidence in Ethiopia highlighted the urgent need for rapid diagnostic methods [9, 10]. However, rapid detection and diagnosis of MD/X/R-TB is less practiced due to shortage of laboratory facilities. In Ethiopia, sputum smear microscopy remains the most common method for diagnosing TB. However, smear microscopy lacks sensitivity and specificity [12]. Culture for Mycobacterium species is not available as routine tests in Ethiopia.Thus, the use of simple, rapid molecular tests to diagnose TB and drug-resistant TB is important. The WHO endorsed the use of the Xpert assay in 2010. The Xpert assay detects simultaneously *M. tuberculosis* and its RIF resistance, which is commonly considered as a marker of MDR-TB [10]. The assay provides results directly from clinical specimens in less than 2 hours [10, 11]. In 2015, most developing countries performed Xpert test for the diagnosis of presumptive MDR-TB and people living with HIV [9]. In the studied region, Xpert test was implemented in 2015.Thus, the purpose of this study was to describe the preliminary report on the implementation of Xpert test in the diagnosis of TB and its RIF resistance at FHRH and DGH hospitals, located about 100km far apart, in Amhara Regional State, Northwest part of Ethiopia.

Materials and methods

Study design, setting and data collection

A retrospective cross-sectional study of 1922 clinically suspected TB patients who have submitted sputum sample for GeneXpert analysis and met the definition of presumptive TB and MDR-TB were included in the study. The study was conducted at FHRH and DTGH, Northwest Ethiopia. These two hospitals are among the busiest hospitals in Northwest Ethiopia that provide referral health services including TB diagnosis and treatment.

All patients who presented from November 2015 to April 2016 and had registered data on their sex, age, HIV status, presumptive MDR-TB status and Xpert results were included for analysis. Data were retrieved in the period of 1-30 April, 2016.

Xpert MTB/RIF testing

Sputum samples were collected and processed directly to Xpert test (Version 4), according to the manufacturer's instructions. The sample reagent was added in a 2:1 ratio (i.e. 1.5ml of bactericidal sample reagent with 0.5ml of specimen) to unprocessed specimens in 15 ml falcon tube and the tube was manually agitated twice during a 15 minute incubation period at room temperature. Then 2 ml of the inactivated material was transferred to the test cartridge by a sterile disposable pipette. Cartridges were loaded into the Xpert assay device and the results were interpreted as previously described [9-11]. Invalid/error results were repeated and the final results were registered. Laboratory staffs in FHRH and DTGH were trained how to use the Xpert modules and cartridges, specimen handling, management of invalid or error results, new recording and reporting tools, and the interpretation of results as per the standard protocol.

Statistical analysis
All data were entered, cleared, and analyzed using SPSS statistical software package version 22. Descriptive statistics was used to determine differences within the data of variables. Associations between Xpert results and patients' age, sex, HIV status and presumptive MDR -TBstatus were determined using Chi square test. A P-value of < 0.05 was considered statistically significant.
Operational definition: According to the standard definitions of the National Tuberculosis and Leprosy Control Program guideline (NLCP) adopted from the WHO [12];

- **Presumptive MDR-TB:** is a diagnosis given to patients with a high risk of MDR-TB and a clinical decision has been made to start MDR-TB treatment before drug sensitivity testing results are available.
- **MDR-TB:** infection caused by bacteria that are resistant to treatment with at least two of the most powerful first-line anti-TB drugs, isoniazid (INH) and rifampicin (RIF).

Ethical issues
Permission and ethical clearance was obtained from Amhara Regional Health Bureau Institutional Review Board (IRB) at Bahir Dar Regional Health Research Laboratory Center to utilize the data. No patient details that may link to the patient identity like names was used and the confidentially was maintained.

Results

A total of 1922 patients were retrospectively included in this study. Among these, 1005 (52.3%) were males. The mean age of patients was 31.1 years with standard deviation of 17.5 years (range from 1- 87 years). Children in the age range of 0-14 years were at 542 (28.2%). Of all the study participants 888 (46.2%) of them were HIV infected (Table 1).

Table 1: Socio-demographic and HIV status of study participants at FHRH and DTGH, 2016

Variables	**Number (%)**	
Sex		
Male	1005 (52.3)	– **Mean 31.1 years**
Female	917 (47.7)	– **SD 17.5**
Total	1922 (100)	– **Min 1**
		– **Max 87**
Age category		
0-14	542 (28.2)	
15-29	308 (16.0)	
30-44	585(30.4)	
45-64	366 (19.0)	
>64	121 (6.3)	
Total	1922 (100)	

HIV status	
Yes	888 (46.2)
No	520 (27.1)
Unknown	514 (26.7)
Total	**1922 (100)**

Overall among the total study participants with presumptive TB cases processed using Xpert test, 258 (14.6%) of them were positive for TB (prevalence was calculated only from valid runs). Of these *M. tb*-positive, RIF-susceptible, *M.tb*-positive, RIF- resistant and *M. tb*-positive, RIF- indeterminate were found to be at 211 (81.8%), 24 (9.3%) and 23 (8.9%) respectively (Table 2).

Table 2: Xpert test result of study participants at FHRH and DTGH, 2016.

	FHRH, % (N)	DTGH, % (N)	Total, % (N)
Number of total samples processed	544	1378	1922
M. tuberculosis-positive, RIF- susceptible	80.9 (76/94)	82.3 (135/164)	81.8 (211/258)*
M. tuberculosis-positive, RIF- resistant	11.7 (11/94)	7.9 (13/164)	9.3 (24/258)**
M. tuberculosis-positive, RIF- indeterminate	7.4 (7/94)	9.8 (16/164)	8.9 (23/258)
M. tuberculosis negative	81.2 (407/501)	87.0 (1100/1264)	85.4 (1507/1765)
Invalid/error results	43	114	8.2 (157/1922)

* Prevalence of TB was calculated from valid runs ((211+24+23)/ (1922-157))
** Prevalence of RIF resistance was calculated from total positive runs (24/ (211+24+23))
In this study**,** around 81.0% (1556/1922) of the suspected TB patients were clinically diagnosed with MDR-TB. In this study, we observed presumptive MDR-TB status among new cases at 1054 (54.8%) followed by the relapse cases at 348 (18.1%) and the treatment after failure at 102 (5.3%) (Table 3).

Table 3: Frequency of presumptive DR-TB based on clinical grounds at FHRH and DTGH, 2016.

	Patient group	**Number**	**%**
Presumptive DR-TB	New case	1054	54.8
	Relapse	348	18.1
	Treatment after lost to follow up	4	0.2
	Treatment after failure	102	5.3
	MDR contact	8	0.4
	Other	40	2.1
	No result	366	19.0
	Total	***1922***	***100.0***

However, among 258 TB positive cases detected using Xpert test only 9.3% (24/258) of them were found to be RIF resistance cases. Among the total RIF cases detected 41.7% (10/24) of the cases were new and

29.2% (7/24) of them were relapsed TB cases. The Xpert test result among presumptive DR-TB groups showed statistical significant difference (p=0.000) (Table 2 and Table4).

Table 4: Xpert MTB/RIF assay result of study participants with variables at FHRH and DTGH, 2016.

Variables		Xpert results						P- value
		*T	RR	TI	N	I	Total	
Sex	Male	127	17	7	773	81	1005	
	Female	84	7	16	734	76	917	0.009
Age category	0-14	31	1	5	461	44	542	
	15-29	53	7	4	208	36	308	
	30-44	69	8	9	462	37	585	0.000
	45-64	42	5	4	282	33	366	
	>64	16	3	1	94	7	121	
	Total	211	24	23	1507	157	1922	
HIV status	Yes	85	8	15	699	81	888	
	No	77	12	5	393	33	520	0.003
	Unknown	49	4	3	415	43	514	
	Total	211	24	23	1507	151	1922	
Presumptive DR-TB	**N	84	10	9	866	85	1054	
	R	66	7	6	240	29	348	
	L	1	0	0	2	1	4	
	F	27	2	2	63	8	102	0.000
	MDR contact	0	1	0	7	0	8	
	Other	6	0	0	33	1	40	
	No result	27	4	6	296	33	366	
	Total	211	24	23	1507	157	1922	

*T: *M. tuberculosis*-positive, RIF susceptible, RR*: M. tuberculosis*-positive, RIF- resistant, TI: *M. tuberculosis*-positive, RIF- indeterminate, N: *M. tuberculosis negative*, I: Invalid/error results
**N: new cases, R: relapse, L: lose to follow up, F: treatment failure

Higher proportion of *M. tb*-positive results were documented among male patients at 58.5% (151/258), in the age group of 30-44 at 33.3% (86/258), new presumptive MDR-TB suspects at 39.9% (103/258) and HIV infected cases at41.9% (108/258). The different Xpert results showed statistical significant difference among the different age groups (p=0.000), sex (p =0.009), HIV status (p=0.003) and presumptive MDR-TB status (p=0.000)(Table 4).

Discussion

Identification and drug resistant testing of *Mycobacterium* species remain a challenge in Ethiopia due to limited laboratory facilities. In the studied area the laboratory diagnosis of TB remains mainly in a stage of Ziehl Nielseen (ZN) smears. However, ZN smear lacks sensitivity. This has implications on wrong patient management, improper use of anti-TB drugs and development of drug resistance [5, 13]. In TB endemic areas like Ethiopia, Xpert test can serve as a sensitive and time saving diagnostic modality for

detection of TB [14]. Moreover, Xpert offers an opportunity for timely and accurate initiation of TB treatment and shortened time of diagnosis in high-burden settings [15, 16]. This study aimed to assess the first implementation report on the diagnosis of TB and its RIF resistance in FHRH and DTGH, Northwest Ethiopia.

In this study, 1922 TB suspected cases had clinical results indicative of TB. Among these, we documented overall prevalence of TB diagnosed using Xpert test at 258 (14.6%). Of which, prevalence of RIF resistance detected using Xpert test was found to be at 9.3 %, which is comparable with previous reports from Northwestern Ethiopia and national wide survey in Ethiopia [17, 18]. Similar findings were also reported from the studies conducted on Xpert test in Ethiopia and elsewhere in the world ranged from 19.4%-45.3% [19-21].On the other hand, it is lower than the finding from Bahir Dar [22]. Another study in Nigeria reported a RIF resistance at 6% [23]. The possible explanation for this difference could be due to the fact that this study was conducted at the site where TB patients less likely served for medical attention and most likely they have accustomed to visit nearby and relatively advanced health institutions. In addition, the design of the studies, factors such as sample size, type and volume of specimen used might be reasons for the discrepancies in Xpert test results.
In this study, TB-HIV co infection rate was at 41.9% (108/258). The WHO report in 2015 estimated a 10% TB/HIV co-infection in Ethiopia [9], which is much lower than the above findings at 41.9%. The HIV infected patients are one of the eligible groups recommended to be tested by the Xperttest for TB and DR-TB [10], this might be the possible reason that could explain the above disparity. However, similar other studies elsewhere in the world have reported at 36.3% of TB/HIV co- infection using the Xpert test [24]. Furthermore, in northern Ethiopia TB/HIV coinfection was reported at 11.4% [25], in Brazil at 39.0% [26], and Western Kenya at 55.5% [27]. Although there is additional information (like CD4 count and treatment use) about the HIV infected individuals in our sample, the fairly high prevalence of TB among HIV/AIDS patients seeks care and prompt treatment.

MDR-TB is more difficult and costly than normal TB to treat, and is more often fatal. Culture based drug susceptibility testing method can provide definitive results, but are labour intensive and time consuming, usually requires at least 14 days for primary isolation of the organism and another 14 days for drug susceptibility test [28]. Furthermore, clinical diagnosis of drug resistance TB is difficult. Thus, molecular methods that target drug resistance are a suitable approach for a rapid drug susceptibility testing [29]. In our study, clinical diagnosis confirmed 81.0% (1556/1922) of the cases as MDR-TB. However, Xpert test detected only 9.3% of the RIF resistance cases. This implies that, the result obtained from the Xpert test in this study has prevented unnecessary treatment of cases, which has great advantage for the patient in terms of avoiding drug toxicity, improper use of anti-TB drugs and development of drug resistance. Furthermore, the Xpert test is contributed to TB prevention and control in the rapid diagnosis and treatment of TB disease and its associated drug resistance. The rapid diagnosis of RIF resistance potentially allows TB patients to start on effective treatment much sooner than waiting for results from other types of drug susceptibility testing. It is also supported by the fact that the information provided by the Xpert test also contributed to cost savings by avoiding unnecessary treatment and aids in selecting appropriate treatment regimens and reaching infection control decisions quickly [30].

Although it was not the main objective of this study, the authors did not use the standard diagnostic techniques to compare Xpert test sensitivity, specificity and predictive values. Hence, the main limitations of our study were the lack of culture result (as no culture facility), chest X- rays and smear result findings which might have determinant factor for comparative performance study. However, our study was the only one report that provides baseline information concerning on the implementation of the Xpert test at FHRH and DTGH.

In conclusion, the study has clearly brought to light that high magnitude of TB and a high prevalence of HIV infection in this TB cohort was documented. Similarly in the studied area, 81.0% patients were presumptively diagnosed with MDR-TB. However, Xpert detected only 9.3% RIF resistance cases. Improving TB detection rates and further reducing the burden of disease in the study site in particular and Ethiopia in general will require optimization of the current laboratory system as well as the introduction of new diagnostic technologies like Xpert test with improved sensitivities and specificities. The Rapid diagnosis of RIF resistance potentially allows TB patients to start on effective treatment at the earliest point of time. Therefore, it is important to sustain and scale up the use of Xpert test for rapid diagnosis of TB and RIF resistance at the study hospitals and other similar health facilities in the region.

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