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RESEARCH ARTICLE

Use of Xpert MTB/RIF for the Identification of TB and Drug Resistance Among Smear-Negative and Re-Treatment Cases in Rural Areas of Ethiopia

Mesfin Worku[1,*], Mulualem Agonafir[2], Mubarek A. Yassin[2], Mohammed A. Yassin[3], Daniel G. Datiko[2], Sally Theobald[4] and Luis E. Cuevas[4]

[1]*School of Medical Laboratory Sciences, College of Medicine and Health Science, Hawassa University, Hawassa, Ethiopia*
[2]*REACH Ethiopia, Ethiopia*
[3]*Global Fund to Fight AIDS Tuberculosis and Malaria, Geneva, Switzerland*
[4]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

Abstract:

Introduction:

Tuberculosis (TB) remains a leading infectious cause of morbidity and mortality worldwide. A key contributor to this burden is poor diagnosis as only 60% of new pulmonary tuberculosis (TB) cases in Africa are ever detected. Therefore, this study aimed to assess the feasibility of Xpert MTB/RIF test implementation in the region, and the performance of the assay to increase case detection on the selected rural health care setting.

Objective:

To assess the feasibility of Xpert MTB/RIF test implementation in the rural health care setting in Southern Ethiopia.

Methods:

Two Xpert MTB/RIF machines were brought in 2012 through TB REACH project. It was placed at Yirgalem hospital and at Aletawondo health centre. The instruments were installed after formal training was provided to laboratory technologists for three days. We collected sputum sample from participants who repeatedly had negative smear microscopy and those who had not responded to first-line anti-TB drugs.

Result:

Of the total participants tested, 1828 have valid result (MTB-, MTB+/RIF-, MTB+/RIF+, MTB+/RIF Indeterminate). From the participants with valid results, 217 (11.9%) were Xpert-positive of which were 165 (9.0%) RIF-negative, 6 (0.3%) RIF-indeterminate and 46 (2.5%) RIF-positive. Among TB suspects with previous treatment history and positive by Xpert, RIF resistance was detected in 10 (2.2%). From the new TB suspects with positive Xpert, RIF resistance was detected in 29 (2.7%). All cases identified were linked with TB/MDR-TB treatment centers.

Conclusion:

Xpert provides an additional tool for the diagnosis of TB and drug resistance, with almost 12% of new and retreatment cases obtaining information that is useful for clinical management. To enhance its efficient utilisation, operational challenges should be minimized particularly in relation to availing robust alternative power source.

Keyword: Xpert, Tuberculosis, SNNPR, Operational challenges, Negative smear microscopy, TB/MDR-TB.

1. INTRODUCTION

Tuberculosis (TB) remains a leading infectious cause of morbidity and mortality worldwide. In 2013, there were 9.0 million new TB cases, 1.5 million TB deaths and over 36% of

* Address correspondence to this author at theSchool of Medical Laboratory Sciences, College of Medicine and Health Science, Hawassa University, Hawassa, Ethiopia; Tel: +251911904261; Emails: mesfinwh@gmail.com

all cases remained undetected. The African Region had approxi-mately one quarter of the world's cases, and the highest rates of cases and deaths relative to its population [1].

A key contributor to this burden of morbidity and mortality is poor diagnosis as only 60% of new pulmonary tuberculosis (TB) cases in Africa are ever detected [2]. The case detection rate of TB in Ethiopia remains low (62 /100000) [1] and this

inadequate case detection reflects, in part, the limitations of the diagnostic used, with sputum smear microscopy remaining the mainstay of TB diagnosis in countries with limited resources [3 - 5].

Accurate and rapid detection of TB and drug resistant-TB is vital to improve patient outcomes, increase the cure rate and decrease mortality [6]. In 2010, WHO endorsed a rapid, automated, cartridge-based nucleic acid amplification test (NAAT), the Xpert MTB/RIF assay (Cepheid, Sunnyvale, USA), that simultaneously detects the presence of TB and genetic markers of rifampicin (RIF) resistance.The assay has high sensitivity (89%) and specificity (99%) for pulmonary TB detection [7].

The Ethiopian TB control program uses a passive strategy to identify cases of TB and patients are mostly identified when they attend curative and diagnostic services. To complement this approach, we have implemented a community-based approach in which health extension workers (HEWs) conduct house to house visits to identify individuals with chronic cough or, collect sputum for examination, which is collected by supervisors and examined at the nearby designated microscopy centre, as described in Yasin *et al.* 2013 [8]. The approach increased case notification and improved treatment uptake.

To optimise the identification of patients, we also implemented the Xpert MTB/RIF platform at two selected sites in the Southern Nations, Nationalities and Peoples Region (SNNPR).

2. MATERIALS AND METHODS

2.1. Settings

Yirgalem Hospital is a tertiary health care hospital serving the population of south nations and nationalities (SNNPR). About 15 million populations are getting service by the hospital from South Nation and Nationalities Region (SNNPR) and the neighbor Oromia region. The hospital is located 317 km south from capital Addis Ababa and 45 km from Hawassa town. In the hospital a Directly Observed Therapy; Short-Course (DOTS) clinic is operating under the National Tuberculosis and LeprosyControl Program (NTLCP) of Ethiopia, under which the diagnosis of pulmonary TB is followed by an examination of three sputum smears (spot morning spot) by Zihel –Nielsen staining method for acid-fast bacilli (AFB). Chest radiographs and pathological investigations are also used to support the diagnosis. Patients diagnosed with tuberculosis are referred to the DOTS clinic where they are registered and treated according to the national TLCP guideline (Ministry of Health of Ethiopia (MOH)

2.2. Study Design

We conducted a prospective study to evaluate the diagnostic ability of Xpert MTB/RIF in identifying patients with pulmonary TB in children with the Human Immunodeficiency Virus (HIV), children, patients who repeatedly had negative smear microscopy and those who had not responded to first-line anti-TB drugs and were suspected to have drug resistance. The study was conducted in 19 rural districts of Sidama zone of the SNNPR with a predominantly farming subsistence population. Two Xpert MTB/RIF machines were brought in 2012 through TB REACH project. The platform was the first of its kind to be used in rural health facility settings of the nation. It was placed at Yirgalem hospital, one of the oldest hospitals found in the country, and at Aletawondo health centre. The hospital serves people of 19 rural districts, two administrative towns and areas bordering the Sidama zone. Similarly, the two machines were assumed to provide service to the Sidama zone and bordering area.

2.3. Training

The instruments were installed after formal training that included theoretical and hands-on practice was provided to laboratory technologists for three days. A separate laboratory register was prepared to simultaneously keep the results and patient details in a hard copy. As a follow-up, frequent supervisions were conducted and on-site refresher trainings were provided on troubleshooting, maintenance and data management.

2.4. Source of Sputum Specimen

We collected sputum sample from patients with pulmonary TB in children with the Human Immunodeficiency Virus (HIV), children, patients who repeatedly had negative smear microscopy and those who had not responded to first-line anti-TB drugs and were suspected to have drug resistance to see detection rate of Xpert MTB/RIF testing machine.

2.5. Study Procedures

Health Extension Workers (HEWs) interviewed and enrolled study presumptive TB patients who have consented using a structured questionnaire for demographic, clinical and epidemiological data that is provided by the national TB control program. Before collecting the first specimen, interviews were conducted by field supervisors.

2.6. Sample Collection and Processing

3–5ml of sputum was spat carefully by each participant into a wide-mouthed, unbreakable, leak-proof container and closed the lid tightly. The sample was placed in re-sealable plastic bag kept in a cold chain and finally transported to the Xpert MTB/RIF testing sites in less than 24 hours.

2.7. Gene Xpert Testing

The Xpert MTB/RIF assay was performed according to the manufacturer's instructions. A sputum sample reagent buffer containing NaOH and isopropanol was added at 2:1 ratio to the untreated sputum ensuring a final volume of at least 2 ml. Manually agitated and kept for 10 min at room temperature, then shaken again and kept for 5 min; 2 ml of the inactivated material was transferred to the cartridge containing the wash buffer, reagents for lyophilized DNA extraction and PCR amplification, and fluorescent detection probes. The cartridge was placed in the test platform of the instrument. Results wereautomatically generated within 2 h and reported as M. TB-negative or - positive (with semi-quantification) and RIF sensitive or resistant.

2.8. Data Analysis

Data was exported from the instrument to an MS Excel sheet and analyzed using SPSS version 20 (SPSS, Inc., Chicago, USA). Proportions were computed and Chi-square test was applied to assess group differences. P values<0.05 were considered significant.

2.9. Ethics Approval

The operational research was conducted under routine National TB Programme and implemented by TB programme coordinators and health workers in the public health sector. Therefore, ethical clearance was not required for the research. However, we have obtained informed consent from the study participants. Study participants who were diagnosed to have TB were started on anti-Tb treatment as per the National Guideline. We have offered advice to patients who were negative by the test to undergo further medical examination [16].

3. RESULTS

From March 2012- April 2014, Xpert MTB/RIF test was utilized to diagnose TB among 2024 presumptive TB cases. The participant composition involved 1098 (54.2%) female, 926 (46.8%) male and 108(5.3%) paediatric presumptive TB cases. Majority of the participants were married (80%), had no formal education 1173 (83.1%) and had the previous history of TB treatment1186 (69.9%)

In addition to chronic cough for 8 weeks and above 1270 (76.3%), the participants had clinical symptoms such as fever 1778 (94.9%), weight loss 1750 (94.3%), night sweating 1820 (97.1%), loss of appetite 1752 (93.6%) and chest pain 1790 (95.3%). From the participants who had x-ray and HIV tests performed, 65 (18.1%) had abnormal x-ray suggestive of TB and 35 (5.2%) were HIV positive, respectively.

Of the total participants tested, 1798 (88.8%) have valid result (MTB-, MTB+/RIF-, MTB+/RIF+, MTB+/RIF Indeterminate). The remaining 226(11.2%) had failed tests (error, invalid or no result). From the participants with valid results, 217 (11.9%) were Xpert-positive of which were 161 (8.0%) RIF-negative, 6 (0.3%) RIF-indeterminate and 45 (2.2%) RIF-positive (Table **1**).

Table 1. Xpert results for the detection of *M. tuberculosis* in Southern Ethiopia.

Xpert result	Frequency	Percent
MTB+/RIF-	161	8.0
MTB+/RIF+	45	2.2
MTB+/RIF Indeterminate	6	0.3
MTB-	1586	78.4
ERROR	168	8.3
INVALID	49	2.4
NO RESULT	9	0.4

Among TB suspects with previous treatment history and positive by Xpert, RIF resistance was detected in 11 (2.0%). From the new TB suspects with positive Xpert, RIF resistance was detected in 34 (2.7%) Table **2**. All cases identified were

linked with TB/MDR-TB treatment centres.

The majority of 169 (79.3%) TB cases detected were in the age group 15-44 years. The number of TB cases detected among participants with no previous TB contact history is twice that of the participants with contact history. Similarly, the number of cases detected among participants with no previous treatment history was twice that of participants with previous treatment history. More males (114) were detected than females (Table **3**).

Table 2. The proportion of Xpert positivity and rifampicin resistance in previously treated cases.

-		Xpert result			
		MTB+/RIF-	MTB+/RIF+	MTB+/RIF Indeterminate	MTB-
Previous treatment history	No	102	34	5	1111
	Yes	59	11	1	475

4. DISCUSSION

Using Xpert has high sensitivity and specificity in detecting MTB in both smear negative and positive among clinical specimens [7, 9, 10] more specifically could be used as a routine method in screening smear-negative patients with high suspicion of TB [9]. We report a high prevalence of MTB+ as high as 11.2% in remote and rural settings in Ethiopia more among women. This was attributable to the reduced barriers to services through community-based intervention [8, 11]. A similar study conducted in Benin supported that The Xpert MTB/RIF test has superior performance for rapid diagnosis of *Mycobacterium tuberculosis* over existing AFB smear microscopy [17]. Another similar study conducted somewhere else indicated that GeneXpert has a higher sensitivity than AFB smear microscopy in respiratory samples. GeneXpert can be a useful tool for early diagnosis of patients with high clinical suspicion of pulmonary tuberculosis [18]. Another study report in Nigeria indicated that Xpert gene assay methods in TB/MDRTB diagnosis in their facility reveal high positive TB and Rifampicin resistance [19].

Furthermore, the majority of the cases were aged between 15-44 years which are economically productive workforce [12].

Ethiopia is one of the five African countries listed as countries with a high number of missed cases [1]. The innovative community-based approach practiced in the SNNP regional state of Ethiopia had demonstrated the detection of more TB cases [8]. Moreover, the introduction of rapid and highly sensitive 'point of care' diagnostic tools such as Xpert could avert events that could occur as a result of the use of less sensitive methods thereby increasing case notification. This study demonstrated that Xpert picks a significant proportion of TB cases in rural settings where AFB smear microscopy is the cornerstone of TB diagnosis and other more sensitive diagnostic tools such as culture are scarce In the current study, Xpert was able to detect 11.9% TB cases (of which 1% children) that were otherwise missed by smear microscopy. Similarly, a study conducted in Pakistan has revealed that Xpert was able to detect 10-15% more cases than ZN microscopy in smear-negative cases [9].

Table 3. TB Risk factors and Xpert positivity among participants.

Characteristics	Values	TB not detected	TB detected	P-value
Previous treatment history (n=1507)	No	956	116	.063
	Yes	375	60	
TB contact history (n=1552)	No	926	113	.067
	Yes	443	70	
HIV (n=559)	Negative	431	94	.593
	Positive	28	6	
Age, Years (n=1741)	<15	81	19	.077
	15-24	153	32	
	25-34	391	55	
	35-44	392	48	
	45-54	305	34	
	55-64	144	18	
	>65	62	7	
Sex (n=1791)	Female	706	98	.365
	Male	873	114	

In this study of 1798 (88.8%) valid results (MTB-, MTB+/RIF-, MTB+/RIF+, MTB+/RIF Indeterminate), 45 (2.2%) were with rifampicin resistance TB. This finding is comparable to a similar study conducted in Nigeria (1.4%) [19]. However, another similar study conducted elsewhere reported a higher rate of rifampicin resistance TB (25.23%) [21]. The probable reason attributed to this variation could be the sample size used and the geographic variation. The majority were initiated on MDR-TB treatment except few who were deceased at the initial stages of the Xpert implementation [6, 13, 14].

In our study, over 75.6% (34/45) of the cases identified as having rifampicin resistance had no history of previous TB treatment and this underlines the importance of rapid resistance testing in patients with no history of TB. This indicated that Xpert MTB/RIF has an added value in detecting MTB and drug resistance that facilitated and supported the community-based intervention in the study site. This is also supported by a study entitled "The implementation of Xpert MTB/RIF assay for diagnosis of tuberculosis "conducted in Nepal [15, 20].

We were not able to corroborate the rifampicin-resistant results with phenotypic drug susceptibility method which is considered as the golden standard. This was the limitation of the current study. Despite the mentioned problems of implementation and limitation of the study, it was possible to demonstrate the usefulness of Xpert in increasing case detection and confirming drug resistance in resource-limited settings such as ours. The findings of this study will be additional input to further optimize the implementation of the tool in other sites by the control program.

CONCLUSION

As a conclusion, Gene Xpert can be a valuable diagnostic tool in patients of suspected Pulmonary Tuberculosis either AFB smear negative or positive due to its rapidity and synchronized detection of Rifampicin resistance especially advantageous in a patient with MDR and HIV associated tuberculosis. However, cost-effectiveness of GeneXpert especially cartridge and realization of continuous supply of electric power in low-income countries like Ethiopia with a high prevalence of tuberculosis needs special emphasis.

LIST OF ABBREVIATIONS

FMoH	=	Federal Ministry of Health of Ethiopia
MDR-TB	=	multidrug resistant tuberculosis
MTB+	=	*Mycobacterium tuberculosis* positive
MTB-	=	*Mycobacterium tuberculosis* negative
RIF-	=	Refampicin

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

We have obtained informed consent from the study participants. Study participants who were diagnosed to have TB were started on anti-Tb treatment as per the National Guideline.

AVAILABILITY OF DATA AND MATERIALS

The data used for the analysis of the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

AUTHORS' CONTRIBUTIONS

DGD, LEC, Mohammed AY and ST conceived the study. Mubarek AY and MAC lead the field work. DGD, LEC, Mohammed AY and MW did the data analyses and write up of the manuscript. All authors have approved the final submitted manuscript.

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