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Use of Xpert MTB/RIF Assay in Rural Health Facilities in Southern Ethiopia

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Abstract

Background: Tuberculosis (TB) is the leading cause of morbidity and mortality in Ethiopia. We describe the performance of the Xpert-MTB/RIF assay for presumptive TB in health facilities that refer samples to a regional reference laboratory.

Methods: A cross-sectional study was conducted in patients with presumptive TB from 1st April 2015 to 30th August 2016. The study was performed in Gambo Hospital, Ethiopia. The samples sent to the referral laboratory were analyzed according to national protocols on the diagnosis of TB and multidrug resistant-TB in children with presumptive TB.

Results: We studied one sample each from 309 unique patients; 197 (63.8%) were less than 14 years old, and 165 (53.4%) were male. The most commonly analyzed sample was gastric aspiration (n=144, 46.6%) followed by sputum (n=92, 29.8%). Gastric aspiration was performed mainly in children (98.6%, 142/144; p<0.001), while peritoneal effusion (94.4%, 17/18; p<0.001), pleural effusion (80.8%, 21/26; p<0.001), lymph node (63.6%, 14/22; p=0.01), and sputum (56/92, 60.9%; p<0.001) were performed mainly in adults. For 10 samples, the results were not available from the referral laboratory. The samples were positive for TB by Xpert MTB/RIF in 22.4% (67/299) of the samples. Only one sample was resistant to rifampicin (0.3%). The results of Xpert MTB/RIF were positive in 76.2% (16/21) of the lymph node samples (p<0.001), 22.3% of the gastric aspiration samples, 20.0% (31/139) of the sputum samples, 1.5% (1/17) of the ascites fluid samples, and 0.0% (0/25) of the pleural effusions (p=0.002).

Conclusion: Xpert MTB/RIF facilitates the diagnosis of TB, with microbiological confirmation, in up to 20% of the samples.

Keywords: Xpert MTB/RIF; Tuberculosis; Gastric aspiration samples

Introduction

The Xpert MTB/RIF assay is considered a great advance over conventional smear for diagnosing tuberculosis (TB) and multidrug resistant (MDR)-TB by simultaneously detecting *M. tuberculosis* and rifampicin resistance bacilli [1]. Multiple studies have consistently shown that Xpert MTB/RIF can identify a substantial proportion of smear-negative TB patients and extra-pulmonary TB (EPTB), particularly in people co-infected with human immunodeficiency virus (HIV) [2]. The World Health Organization endorsed the Xpert MTB/RIF assay in 2010 for use in resource-limited countries with an important prevalence of TB as a first line, rapid diagnostic test for TB in HIV-infected patients and for managing suspicion of MDR-TB [3].

Ethiopia ranks eighth in the list of the 30 high TB burden countries [4]. Ethiopia is one of 21 recipient countries to implement the TBXpert Project. The Federal Democratic Republic of Ethiopia Ministry of Health/ Ethiopian Public Health Institute followed the Implementation Guideline for GeneXpert MTB/RIF Assay in Ethiopia in June 2014. This is the main guiding document for program coordinators, National and Regional Reference Laboratories, regional health boards, health facilities, academic and research institutions, and other stakeholders involved in TB and HIV programs [5]. Ethiopian researchers have described different aspects of the program [1,6-10], mostly

focusing on settings where the healthcare facilities and the reference laboratories share a location, but there are few studies in places where these facilities are separate [11]. Know the proper working of samples transport to referral laboratory must be useful to improve the application of the procedure to other remote areas in Ethiopia.

The present study aims to describe the performance of the Xpert MTB/RIF assay in people with suspected tuberculosis in health facilities that refer the samples to a regional reference laboratory.

Material and Methods

Cross-sectional study in patients with suspected TB to evaluate the performance of Xpert MTB/RIF assay for detection of MTBC. The study was performed at Gambo Rural Hospital (GRH) in Gambo town, 18 km from Arsi Negele (main town) and 45 km from Kuyera Hospital (the reference hospital for West-Arsi Province, population of 2,637,657, and a population density of 133.05); both towns are located on the highway connecting Addis Ababa and Kenya. The catchment area of the GRH is restricted to approximately 100,000 inhabitants. Most of the population live in a rural setting and work in agriculture and farming. The patients came from far to be diagnoses in GRH. In previous studies four out of five TB cases diagnosed at GRH were transferred out to star the treatment [12]. Gambo town is 200 km from Adama Hospital (the reference hospital for the Oromia Region of Ethiopia), and 250 km from Addis Ababa, the capital city.

The samples sent to the referral laboratory were analyzed according to national protocols [3]. One of sample was storage for Xpert MTB/RIF assay (at 2 to 8°C up to 5 days). The sample was transported use triple packaging and the specimen reached the testing site within 5 days after collection. Cold chain was maintained using an ice pack and transported to referral laboratory. The Xpert assay was the primary test for diagnosing TB and MDR-TB in presumptive MDR-TB cases as well as TB in HIV-positive presumptive TB. Xpert was also used as one of several diagnostic tests to diagnose EPTB in presumptive TB (on cerebrospinal fluid [CSF], lymph node [LN] aspirates by fine needle aspiration, biopsy, and pus samples), and to diagnose MDR-TB in children with presumptive TB (using gastric aspirate, induced sputum, LN aspirate, pus, CSF, or biopsy). Only one sample for patients was processed.

The samples were sent to the referral laboratory according to national protocols [5]. The samples were treated following the manufacturer's instructions, transferred into the cartridge, and loaded into the GeneXpert instrument, with the remaining assay steps completed via an automated process [13].

Statistical Analysis

We used SPSS software for the statistical analysis. In the descriptive study, we expressed continuous variables as the mean ± standard deviation (SD) and qualitative variables as relative frequencies. We used the Chi-squared test to assess the statistical association between the type of sample used, the results of the Xpert MTB/RIF assay, and the age of the patient. AP value of less than 0.05 was considered to indicate statistical significance.

Ethical Approval

Ethics committee approvals were obtained from both the local Research and Publication Committee of the Gambo General Hospital and the Health Unit and Ethical Review Committee of the Ethiopian Catholic Secretary (GH/MSMHF/709). Oral informed consent was recorded in each questionnaire, and for minors/children enrolled in this study, consent was obtained from the next of kin, caretakers, or guardians. We did not obtain written consent because there is a high rate of illiteracy in the population.

Results

During the study period, we studied a single sample in each of 309 unique patients: 197 (63.8%) were less than 14 years old (from 1 month to 13 years), while 112 (36.2%) were 14 years or older (from14 years to 78 year); 165 (53.4%) patients were male. The most commonly analyzed samples were of gastric aspiration (n=144, 46.6%), followed by sputum (n=92, 29.8%), pleural effusion (n=26, 8.4%), lymph node (n=22, 7.1%), peritoneal effusion (n=18, 5.8%), CSF (n=4, 1.3%): purulent abscess (n=2, 0.6%) and joint effusion (n=1, 0.3%).

Gastric aspiration samples were obtained mainly from children (98.6%, 142/144; p<0.001), while adults contributed more samples from pleural effusion (80.8%, 21/26; p<0.001), peritoneal effusion (63.6%, 17/18; p<0.001), and sputum (60.9%, 36/92; p<0.001) and LN (63.6%, 14/22; p=0.01).

For 10 samples, the results were not available from the referral laboratory. The samples tested positive for TB by Xpert MTB/RIF in 22.4% (67/299) of the total, with similar results in children (23.3%, 44/145) and adults (20.9%, 23/110). Only one sample was resistant to rifampicin (0.3%) **(Table 1)**.

The results of Xpert MTB/RIF were positive in 22.3% (31/139) of the gastric aspiration samples 20.0% (18/72) of sputum samples, and in 1.5% (1/17) of ascites fluid. Samples of LN testing positive were significantly more common according to the Xpert MTB/RIF (76.2%, 16/21; p<0.001). None of the pleural effusion samples were positive (p<0.002).

Discussion

This is a pilot utility study in a rural area, assessing the Xpert MTB/RIF assay as performed according to national guidelines in Ethiopia. The mechanisms for sending the samples to a main referral laboratory work properly.

Gastric aspirate was a useful sample to identify *M. tuberculosis* in children, as 22.3% of samples sent to the laboratory were positive cases. Our rate of *M. tuberculosis* detection was similar as in previous studies performed by Xpert MTB [14,15]. The main limitation was that only one gastric aspirate sample was obtained per patient (if the analysis includes two samples collected on two consecutive days, the

positive rate of *M. Tuberculosis* detection by molecular technique increases). Had samples of induced sputum samples also been collected and analysed on two consecutive days, the detection rate may have been higher still [15], although other authors such as Bunyavasi et al. [16] argue that one sample type is sufficient, reporting that the sensitivity of the Xpert MTB/RIF assay does not differ significantly between sample types in young children with suspected pulmonary TB.

Table 1 Type of sample by age and Results of Xpert MTB/RIF Assay by type of sample.

	Total	Samples							Age group	
		Gastric aspiration	Sputum	Pleural Effusion	Peritoneal Effusion	Lymph node	CSF	Others#	<14 year old	≥ 14 years old
Samples collected by age, n (%)										
Samples sent	N=309	N=144	N=92	N=26	N=18	N=22	N=4	N=3	—	—
<14 year old	197 (63.8)	142 (98.6)*	36 (39.1)	5 (19.2)	1 (5.6)	8 (36.4)	3 (75)	2 (66.7)	—	—
≥ 14 years old	112 (36.2)	2 (1.4)	56 (60.9)*	21 (80.8)*	17 (94.4)*	14 (63.6)**	1 (25)	1 (33.3)	—	—
Results of samples analyzed, n (%)										
Samples with results	N=299	N=139	N=90	N=25	N=17	N=21	N=4	N=3	N=189	N=110
Negative	232 (77.6)	108 (77.7)	72 (80)	25 (100)	16 (94.1)	5 (23.8)	3 (75)	3 (100)	145 (62.5)	87 (79.1)
Positive	67 (22.4)	31 (22.3)	18 (20)	0 (0)**	1 (5.9)	16 (76.2)**	1 (25)	0 (0)	44 (23.3)	23 (20.9)
95% CI of positive results	18.1-27.5	16.2-29.9	13.0-29.4	0-13.3	1.1-26.0	54.9-89.4	4.6-69.9	0-56.1	17.8-29.8	14.4-29.4

Abbreviation: CSF: central spine fluid; CI: confidence interval

#Other samples: 2: purulent samples and one joint effusion

P value: *p<0.001; and ** p=0.01

More than 20% of the sputum samples analyzed tested positive. This result is high, perhaps because the sputum sent to the laboratory came from people suspected of having resistant TB and from HIV-infected patients.

The detection from LN samples was much higher, with more three of five samples testing positive. These findings are consistent with previous reports demonstrating that the accuracy of the Xpert MTB/RIF assay in LN samples for diagnosing TB adenitis eliminates the need for cytology or conventional smear microscopy [10,17,18]. These procedures have been used as the initial diagnostic tools for TBL in low-income countries [17,18].

The accuracy of the test was lower in our study for peritoneal and pleural effusion samples, which is also consistent with other studies performed in low-income countries [19,20].

Indeed, it is well known that diagnosing abdominal tuberculosis from ascitic fluid samples and pleural tuberculosis from pleural samples using routinely available diagnostic methods is challenging due to the paucibacillary nature of the disease. In a recent systematic review investigating Xpert MTB/RIF for the diagnosis of tuberculous pleural effusion, Xpert MTB/RIF showed low sensitivity but excellent specificity [19]. Diagnostic yield of the Xpert MTB/RIF assay on ascitic fluid samples is low and inferior to mycobacterial culture [20].

In our study, only one case was a resistant sample, which is much lower than the prevalence reported elsewhere [19,20].

This study has several limitations: we did not perform a direct diagnostic accuracy test comparing Xpert MTB/RIF and culture (Löwenstein–Jensen medium or mycobacteria growth indicator tube [MGIT]). Likewise, we did not properly record the time taken to send the result to the patients after obtaining the results in the hospital or the characteristics of the sputum (purulent, mucopurulent, and bloody, highly purulent). We are currently working to improve this system in our local protocol.

Conclusions

Multiple studies have consistently shown that Xpert MTB/RIF can identify a substantial proportion of smear-negative TB and EPTB, particularly in people co-infected with HIV. Accurate diagnosis and early treatment of TB has the potential to reduce morbidity and mortality associated with TB. So in our hospital, the Xpert MTB/RIF assay is a very useful tool for improving diagnostic accuracy in TB, even considering that the samples need to be sent to a reference hospital. Moreover, our findings suggest that Xpert MTB/RIF on gastric aspirates is a useful test for the rapid diagnosis of pediatric pulmonary tuberculosis in primary care, so Xpert MTB/RIF on LN samples is useful for diagnosis of TB adenitis and the accuracy of the test is lower for peritoneal and pleural effusion samples.

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Conflict of Interest

There are no conflicts of interest of any kind, including any relationship or dual interest, financial or otherwise, that may affect professional judgment in relation to this article.

References

1. Geleta DA, Megerssa YC, Gudeta AN, Akalu GT, Debele MT, et al. (2015) Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in sputum specimens in remote health care facility. BMC Microbiol 15: 220.
2. Blakemore R, Story E, Helb D, Kop J, Banada P, et al. (2010) Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol 48: 2495-2501.
3. World Health Organization (2011) Policy Statement: Automated real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System. Geneva, Switzerland: World Health Organization.
4. World Health Organization (2017) Global tuberculosis report 2016. Geneva, Switzerland.
5. Federal Democratic Republic of Ethiopia Ministry of Health/ Ethiopian Public Health Institute performs (2014) Implementation Guideline for GeneXpert MTB/RIF Assay. Addis Ababa, Ethiopia.
6. Balcha TT, Sturegård E, Winqvist N, Skogmar S, Reepalu A, et al. (2014) Intensified tuberculosis case-finding in HIV-positive adults managed at Ethiopian health centers: diagnostic yield of Xpert MTB/RIF compared with smear microscopy and liquid culture. PLoS One 9: e85478.
7. Biadglegne F, Tesfaye W, Sack U, Rodloff AC (2013) Tuberculous lymphadenitis in Northern Ethiopia: in a public health and microbiological perspectives. PLoS One 8: e81918.
8. Bezabih M, Apers L, de Jong BC, Rigouts L (2014) GeneXpert MTB/RIF Assay for the Diagnosis of Tuberculous Lymphadenitis on Concentrated Fine Needle Aspirates in High Tuberculosis Burden Settings. PLoS One 10: e0137471.
9. Adelman MW, Tsegaye M, Kempker RR, Alebachew T, Haile K, et al. (2015) Intensified tuberculosis case finding among HIV-infected persons using a WHO symptom screen and Xpert(®) MTB/RIF. Int J Tuberc Lung Dis 19:1197-1203.
10. Tadesse M, Aragaw D, Rigouts L, Abebe G (2016) Increased detection of smear-negative pulmonary tuberculosis by GeneXpert MTB/RIF® assay after bleach concentration. Int J Mycobacteriol 5:211-218.
11. Geleta DA, Megerssa YC, Gudeta AN, Akalu GT, Debele MT, et al. (2015) Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in sputum specimens in remote health care facility. BMC Microbiol. 15: 220.
12. Ramos JM, Tesfamariam A, Balcha S, Biru D, Reyes F, et al. (2013) Management and transference of patients diagnosed with tuberculosis in a rural hospital in Southern Ethiopia. Int J Mycobacteriol 2: 79-83.
13. Cepheid (2011) GeneXpert Dx System Xpert MTB/RIF assay manual: Operator Manual Software Version 4.
14. Hasan Z, Arif F, Shakoor S, Mehnaz A, Akber A, et al. (2016) Effective testing for pulmonary tuberculosis using Xpert MTB/RIF assay for stool specimens in immunocompetent Pakistani children. Int J Mycobacteriol 1: S8-S9.
15. Singh S, Singh A, Prajapati S, Kabra SK, Lodha R, et al. (2015) Delhi Pediatric TB study group Xpert MTB/RIF assay can be used on archived gastric aspirate and induced sputum samples for sensitive diagnosis of paediatric tuberculosis. BMC Microbiol 15: 191.
16. Bunyasi EW, Tameris M, Geldenhuys H, Schmidt BM, Luabeya AK, et al. (2015) Evaluation of Xpert® MTB/RIF Assay in Induced Sputum and Gastric Lavage Samples from Young Children with Suspected Tuberculosis from the MVA85A TB Vaccine Trial. PLoS One 10: e0141623.
17. Wright CA, van der Burg M, Geiger D, Noordzij JG, Burgess SM, et al. (2008) Diagnosing mycobacterial lymphadenitis in children using fine needle aspiration biopsy: cytomorphology, ZN staining and auto- fluorescence—making more of less. Diagn Cytopathol 36: 245-251.
18. Ghariani A, Jaouadi T, Smaoui S, Mehiri E, Marouane C, et al. (2015) Diagnosis of lymph node tuberculosis using the GeneXpert MTB/RIF in Tunisia. Int J Mycobacteriol 4: 270-275.
19. Sehgal IS, Dhooria S, Aggarwal AN, Behera D, Agarwal R (2016) Diagnostic Performance of Xpert MTB/RIF in Tuberculous Pleural Effusion: Systematic Review and Meta-analysis. J Clin Microbiol 54: 1133-1136.
20. Rufai SB, Singh S, Singh A, Kumar P, Singh J, Vishal A (2017) Performance of Xpert MTB/RIF on Ascitic Fluid Samples for Detection of Abdominal Tuberculosis. J Lab Physicians 9: 47-52.

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