Asian Pacific Journal of Tropical Disease

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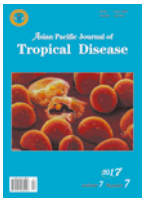

Original article https://doi.org/10.12980/apjtd.7.2017D6-442

Evaluation of GeneXpert MTB/RIF and line probe assay for rapid diagnosis of *Mycobacterium tuberculosis* in Sudanese pulmonary TB patients

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ARTICLE INFO

Article history:
Received 12 Dec 2016
Received in revised form 30 Jan 2017
Accepted 18 Apr 2017
Available online 7 Jul 2017

Keywords:
Mycobacterium tuberculosis
GeneXpert
Line probe assay
Sudan

ABSTRACT

Objective: To study the sensitivity and specificity of line probe assay (LPA) and GeneXpert compared with drug susceptibility test (DST).
Methods: A cross-sectional study was conducted for 126 TB patients in Sudan. *Mycobacterium tuberculosis* was identified by drug susceptibility test. The smear-positive sputum specimens were screened for rapid detection of resistance to rifampicin (RIF) and isoniazid (INH) by molecular LPA and GeneXpert assay.
Results: 67.5% of patients were male, 19.8% were new cases, 57.1% were previously treated TB patients, and 64.3% of the affected patients were in the age group of 16–30 years. About 32.5% of samples were MDR by both DST and LPA, 66.7% were sensitive by both DST and LPA, and 0.79% were MDR by DST and sensitive by LPA. However, 36.51% of samples were MDR by both DST and GeneXpert, and 63.49% were sensitive by both DST and GeneXpert. There was no significant difference between DST and LPA technique ($P = 0.50$). The sensitivity, specificity, positive predictive value and negative predictive value of LPA were 97.6%, 100%, 97.6%, and 100%, respectively; while for GeneXpert, they were 100%, 100%, 100% and 100%, respectively. The RIF resistance was associated with mutation in the region of rpoB 530-533, mostly S531L mutation, and the most INH resistant samples (98.58%) were linked with *KatG* gene and codon 315 (S315T1).
Conclusions: The molecular assays are the best screening tools for MDR TB, which may exceed TB diagnoses.

1. Introduction

Tuberculosis (TB) is the most important infectious disease in the world causing morbidity and mortality among adults[1]. In 2012, 8.6 million people developed TB for the first time, and 13% had TB with HIV infection[2]. Similarly, in 2012, 1.3 million people died from TB, and 320 000 deaths of TB patients were HIV positive[2]. Multi-drug-resistant tuberculosis (MDR-TB) is defined as tuberculosis resistant to at least two main first-line drugs isoniazid (INH) and rifampicin (RIF)[3]. The resistance to the two first-line anti-TB drugs has emerged as a serious threat to global health[4]. Molecular line probe assay (LPA) (Genotype MTBDRplus) has been recently approved for use in low income areas and can be used to screen smear-positive sputum specimens for rapid detection of rifampicin and isoniazid resistance in 1–2 days. Because of the high-risk transmission from person to person, emergence of MDR-TB and extensive drug resistant tuberculosis, the rapid detection of *M. tuberculosis* and rifampicin (RIF) resistance in infected patients is essential for disease management. Culture is the gold standard for final determination, but it takes 2 to 8 weeks. Although smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity and a poor positive predictive value (PPV). Rapid identification is essential to initiate early treatment, improve patient's outcomes, and more effective for public health interventions[5]. Therefore, molecular

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

assays have been used to predict drug resistance in clinical specimens within one working day and are potentially the most rapid methods[6-10]. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device that performs sample processing and semi-nested real-time PCR analysis in a single hands-free step for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens[4,10]. The MTB/RIF assay detects *M. tuberculosis* and RIF resistance by PCR for 81 bp of the *M. tuberculosis rpoB* gene and subsequently probes this region for mutations that are associated with RIF resistance. The assay can generally be completed in less than 2 h[6,9]. The aim of the present study was to determine the sensitivity and specificity of the GeneXpert MTB/RIF assay for detection of resistance pattern of rifampacin, and line probe assay (Genotype MTBDR plus) as a rapid detection method for rifampacin and isoniazid resistance. The results obtained by the molecular assays were compared with the results of culture and drug susceptibility test.

2. Materials and methods

2.1. Study design and setting

A cross-sectional study was conducted at the National Reference TB Laboratory, National Public Health Laboratory, Khartoum, Sudan. A total of 126 specimens of suspected TB patients were collected from December 2011 to March 2015. All specimens showed acid fast bacilli (AFB) microscopically.

2.2. Sample procedure

The early morning deep coughed sputum specimens were collected in sterile containers from all participants after obtaining the written informed consent. Each sample was examined microscopically using Ziehl-Neelsen (ZN) staining[11], then the specimens that showed AFB microscopically were divided into two groups, one for GeneXpert MTB/RIF assay and line probe assay, and the other group for culture. Sputa were decontaminated according to Petroff's method and aliquot of 0.1 mL was incubated at 37 ℃ on Lowenstein-Jensen medium (LJ) and then weekly tested for presence of the growth on LJ medium[12]. The strains which were identified as *M. tubercu1osis* complex were tested for their susceptibility to isoniazid and rifampcin.

2.3. GeneXpert procedure

Briefly, the reagent was added at 2:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during incubation period for 15 min at room temperature. The reagent sample mixture was transferred to the Xpert test cartridge. The cartridge was inserted into the GeneXpert device and the results generated automatically were read after 90 min[7].

2.4. Drug susceptibility test (DST)

DST was performed on the culture to identify *M. tuberculosis* complex (MTBC) strains.

2.5. Line probe assay (LPA)

Line probe assay was performed in three separate rooms, according to WHO recommendations[13]. Five hundred microlitres of processed specimen was used to perform the Genotype MTBDRplus (Hain Life science GmbH assay). Residual processed specimens were refrigerated at 2–8 ℃ overnight after DNA extraction to repeat the test if required.

2.6. Ethical approval

The study protocol was performed according to the Helsinki declaration and approved by the Faculty of Medical Laboratory Sciences, Ethic Committee of University of Khartoum. Informed written consent was obtained from each patient.

2.7. Statistical analysis

Statistical analysis was performed by using SPSS software for Windows (version 16.0).

3. Results

Approximately 67.5% of the patients were male, 64.3% of the affected patients were in the age group of 16–30 years. In the study, 57.1% of cases were previously treated, 19.8% were new cases and 23.1% were unknown (Table 1).

Table 1
Basic data for the study population.

Traits		*n*	Percentage
Age group	⩽ 15	1	0.8%
	16–30	81	64.3%
	31–45	23	18.2%
	46–60	15	11.9%
	⩾ 60	6	4.8%
Gender	Male	85	67.5%
	Female	41	32.5%
Patient case	New	25	19.8%
	Previouslu treated	72	57.1%
	Unknown	29	23.1%

Among 126 RIF resistant isolates, missing WT (wild type) along with known mutations were detected in 38 isolates (30.2%). The RIF mutation was detected in codon S531L (28/126; 22.2%) followed by D516V mutation (6/126; 4.8%), H526Y mutation (5/126; 3.9%) and H526D mutation (0/126). Missing wild types with mutant probe among *katG* were found in 37 isolates (29.4%). Among 126 INH resistant isolates detected by MTBDRplus, *katG* mutations were found in 72 isolates (57.1%). Mutations in codon

S315T1 were detected in 35 INH resistant isolates (27.8%) or 35 of 72 (48.6%) *katG* mutants. Missing wild types with mutant probe among *InhA* were found in 1 isolate (0.79%), and mutations in *InhA* C15T were found in 1 INH resistant isolates (0.79%) (Table 2).

Table 2

Pattern of gene mutations in MD -M. tuberculosis detected by Line Probe Assay (LPA).

Gene	Band	Gene region or mutation	MDR strain
rpoB	WT1	506-509	
	WT2	510-513	
	WT3	513-517	1
	WT4	516-519	
	WT5	518-522	1
	WT6	521-525	2
	WT7	526-529	5
	WT8	530-533	29
	MUT1	D516V	6
	MUT2A	H526Y	5
	MUT2B	H526D	
	MUT3	S531L	28
katG	WT	315	37
	MUT1	S315T1	35
	MUT2	S315T2	
InhA	WT1	15/16	1
	WT2	8	
	MUT1	C15T	1
	MUT2	A16G	
	MUT3A	T8C	
	MUT3B	T8A	

In the present study, 126 smear-positive sputum specimens were tested. The resistant pattern using LPA revealed that 41 isolates (32.5%) were MDR (Figure 1) and 85 (67.5%) were sensitive, and the resistant pattern using GeneXpert showed 46 (36.5%) rifampacin resistant isolates (Figure 2) and 80 (63.5%) rifampacin sensitive isolates. The clinical isolates of *M. tuberculosis* were subjected to conventional DST, and the result showed that 42 (33.3%) were MDR and 84 (66.7%) were sensitive.

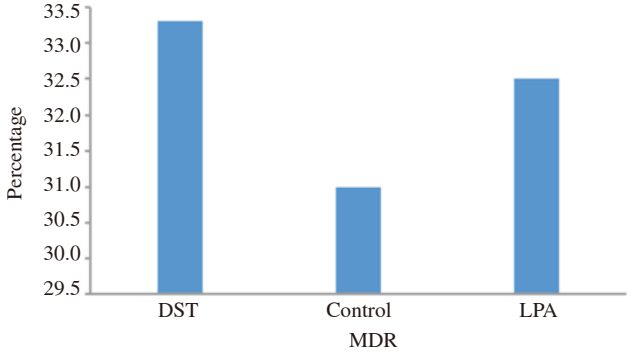

Figure 1. The multi-drug resistance test.

The results of LPA and GeneXpert were compared separately with that of DST (gold standard method), which showed that for LPA, the sensitivity was 92.9%, specificity was 97.6%, positive predictive value was 95.2% and negative predictive value was 96.4%. For GeneXpert, the sensitivity was 100%, specificity was 100%, positive predictive value was 100% and negative predictive value was 100%.

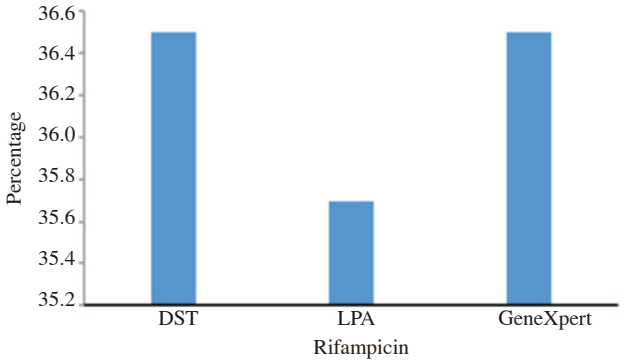

Figure 2. The rifampicin resistance in drug susceptibility testing, line probe assay and GeneXpert.

DST showed that 70 (55.6%) of specimens were sensitive to INH and RIF, 42 (33.3%) specimens were MDR, 4 (3.17%) specimens were sensitive to INH and resistant to RIF and 10 (7.9%) specimens were sensitive to RIF and resistant to INH. According to DST results, most of MDR cases were previously treated [37 (88.2%)], while 4 (9.5%) were new cases and 1 (2.3%) were unknown cases. GeneXpert and LPA are considered as rapid molecular tool with high accuracy for the detection of rifampcin resistant and MDR-TB.

4. Discussion

In the present study 19.8% of the TB patients were newly diagnosed for the first time, while 57.1% had history of TB reflecting the active transmission of TB. About 82.6% of TB patients were in the age group of 16 to 45 years. A previous study in Sudan revealed that 82% of TB patients aged under 50 years old[14]. In the developed countries, the most TB cases were found in Europe; it was more prevalent in elder people due to diabetes mellitus and among immunocompromised patients[15]. The present study revealed high prevalence of MDR among new cases and retreated cases which gives an indication of the presence of a serious problem attributed to either mismanagement of TB patients, wrong diagnosis, delay in diagnosis, wrong or interrupted treatment and mistreatment with both first and second line drugs.

The molecular LPA (Genotype MTBDRplus) was used to screen smear-positive sputum specimens for rapid detection of rifampicin and isoniazid resistance in 1–2 days. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device for the diagnosis of TB and rapid detection of RIF resistance in smear-positive and smear-negative pulmonary and extra pulmonary specimens obtained from presumptive TB patients in 2 h. In the present study, RIF resistance was associated with mutation in the region of *rpoB* 530-533, mostly S531L mutation. Similar results were obtained in Sudan[14], South Africa[16] and Switzerland[17], which found that this mutation was more frequent in MDR-TB strains. The present findings provide the basis for rapid detection of rifampicin resistance. In addition, most INH resistant samples (98.58%) in this study were linked with *katG* gene, codon 315 (S315T1) as indicated in many high TB burden countries[18].

The study indicated that the molecular techniques were highly

consistent with the conventional culture and DST method. Our results showed that 33.3%, 32.5% and 36.5% of samples were MDR when tested by DST, LPA and GeneXpert, respectively. The sensitivity, specificity, positive predictive value and negative predictive value of LPA were 97.6%, 100%, 97.6%, and 100%, respectively. These results were in agreement with the previous studies in Sudan[14] which revealed that the sensitivity and specificity of LPA and DST were 98.3% and 100%, respectively. Similar results were reported in studies in South Africa and Bangladesh[16,19], which compared the result of LPA with the DST and found high sensitivity (95.5%–98.8%) and high specificity (96.9%–100%) of LPA for MDR-TB detection. In the present study the sensitivity and specificity of GeneXpert were 100% and 100%, respectively. Similar results were reported in Greece[20]. However, previous studies indicated that the sensitivity of the MTB/RIF test for detecting RIF resistance was 94.4%–100% and the specificity was 98.3%–100%[21-23]. In addition, our study showed that there is no significant difference in sensitivity and specificity between GeneXpert and LPA and the golden method DST. Finally we conclude that the GeneXpert and LPA were accurate techniques for screening MDR-TB, and reduce the time for diagnoses.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors acknowledge the staff of the National Tuberculosis Reference Lab and Tropical Medicine Research Institute for their support. This research was funded by the National Tuberculosis program, Sudan.

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