International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 02(B); February 2019; Page No. 17248-17250
DOI: http://dx.doi.org/10.24327/ijcar.2019.17250.3224

Research Article

EVALUATION OF THE GENEXPERT MTB/RIF ASSAY FOR RAPID DIAGNOSIS OF TUBERCULOSIS AND DETECTION OF RIFAMPIN RESISTANCE IN PULMONARY AND EXTRA PULMONARY SPECIMENS

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ARTICLE INFO

Article History:
Received 13th November, 2018
Received in revised form 11th December, 2018
Accepted 8th January, 2018
Published online 28th February, 2019

Key words:
Formaldehyde, thyroid, exposed, groups, divided, method, function, individuals, formaldehyderesult, Affected, square, whereas, hypothyroidism, people, methods, dysfunction, pathology, departments, Anatomy, chemical

ABSTRACT

Mycobacterium tuberculosis remains one of the most significant morbidity and mortality. The rapid diagnosis of tuberculosis and detection of Rifampin (RIF) resistance are essential for early disease management. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens. We determined the performance of the MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in smear-positive and smear-negative pulmonary and extra pulmonary specimens obtained from possible tuberculosis patients.

INTRODUCTION

Mycobacterium tuberculosis remains one of the most significant causes of morbidity and mortality from an infectious agent. The incidence of pulmonary tuberculosis in India is nearly 23 to 25 per 100,000 populations, according to the 2015. The proportion of multidrug-resistant (MDR) tuberculosis (TB) cases among new cases is 3 to 4 %, that among previously treated cases is 15.5%, and that among all TB cases is 4.9%). The rapid detection of *M. tuberculosis* and rifampin (RIF) resistance in infected patients is essential for disease management, because of the high risk of transmission from person to person and emergence of MDR-TB and extensively drug resistant tuberculosis. Culture is the "gold standard" for final determination, but it is slow and may take up to 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). Thus, rapid identification, which is essential for earlier treatment initiation, improved patient outcomes, and more effective public health interventions, relies on nucleic acid amplification techniques.

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Collectively, DNA sequencing studies demonstrate that more than 95% of RIF-resistant *M. tuberculosis* strains have a mutation within the 81-bp hot spot region of the *rpoB* gene. Several molecular methods have been developed in recent years for the diagnosis of tuberculosis and rapid detection of drug resistance in clinical specimens, including line probe assays (GenoType MTBDRplus [Hain Life science GmbH, Nehren, Germany], INNO LIPA Rif.TB [Innogenetics, Ghent, Belgium]) and real-time PCR (GeneXpert MTB/RIF; Cepheid, Sunnyvale, CA). Molecular assays have been established to allow the prediction of drug resistance in clinical specimens within 1 working day and are potentially the most rapid methods for the detection of drug resistance. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device that performs sample processing and hemi 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 (3, 9). The MTB/RIF assay detects *M. tuberculosis* and RIF resistance by PCR amplification of the 81-bp fragment of the *M. tuberculosis rpoB*gene and subsequent probing of this region for mutations that are associated with RIF resistance. The assay can generally be completed in less than 2 h

Aim

The aim of this study was to determine the sensitivity and specificity of the MTB/RIF assay for the diagnosis of tuberculosis and rapid detection of rifampin resistance in smear-positive and smear-negative pulmonary and non pulmonary clinical specimens. The results obtained by the MTB/RIF assay were compared with the results obtained by culture and phenotypic susceptibility testing.

MATERIALS AND METHODS

Inclusion criteria

1. All sputum positive cases
2. Sputum negative and clinically positive cases
3. Extra pulmonary TB cases

Clinical samples

In this study, pulmonary and extra pulmonary samples obtained during the clinical routine and sent to the RNTCP i.e. the District TB centre Asaripallam from Kanyakumari Govt Medical College, Asaripallam, Govt hospitals of Kanyakumari dist, PHCs of Kanyakumari Districts. A total of 100 pulmonary specimens (sputum, bronchoalveolar lavage, bronchoscopic aspirate, postbronchoscopic sputum, and gastric fluid specimens) and 100 extra pulmonary samples are going to be tested by Gene Xpert and Microscopy.

Identification of Rifampicin resistance stains will also be identified. MTB/RIF assay:The MTB/RIF assay was performed as described previously. Briefly; sample reagent was added at a 3:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during a 15-min period at room temperature, before 2 ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) was transferred to the test cartridge. All specimens that were culture positive and MTB/RIF assay negative and specimens that were culture negative and MTB/RIF assay positive were retested twice. The last result was used for the analysis.

***Patient groups*:** Patients were grouped as (i) those with smear positive tuberculosis; (ii) those with smear-negative, clinically suspected tuberculosis; (iii) those who were smear positive for tuberculosis but who were nonetheless treated for tuberculosis on the basis of clinical, pathological, and/or radiological findings (clinical tuberculosis); and (iv) those with no bacteriological, clinical, pathological, or radiological evidence of tuberculosis (no tuberculosis). Statiscal analysis of Gene Xpert Positive according to age group with relative to Microscopy positive.

Sensitivity and specificity of MTB/RIF assay.

Detection of rifampin résistance. Susceptibility testing was performed for all the Sputum positive and Sputum negative patients. The MTB/RIF test was positive for 35 of 35 sputum positive samples and 32 out of 5042 sputum negative cases from clinical tuberculosis cases. The sensitivity of microscopy with CBNAAT is found to be 100 percent. The MTB/RIF assay was positive for all of the smear-positive specimens, the sensitivity of the MTB/RIF test, which was as rapid as smear, was much higher than that of smear.

Cbnaat Report of 2018

Study Group: All the samples of District Tuberculosis centre, Kanyakumari district

Study Period: January 2018 to December 2018

Method of Study: Sputum microscopy and CBNAAT

Month	Sputum Positive cases	CBNAAT report	Sputum negative cases Suspected clinical positive cases	CBNAAT Report
January	3	3	245	02
February	3	3	297	02
March	2	2	309	06
April	1	1	310	06
May	5	5	469	04
June	4	4	458	0
July	5	5	688	04
August	1	1	327	01
September	2	2	432	03
October	2	2	438	03
November	3	3	569	01
December	4	4	500	0
	35	35	5042	32

Analysis

The data was tabulated above. The sputum microscopy cases and sputum microscopy negative but clinically positive cases are tabulated separately.

All sputum positive cases numbering 35 were positive in CBNAAT.

Sputum negative cases numbering 5042 cases but suspected clinically positive cases are tested CBNAAT. Out of 5042 cases were tested, 32 were CBNAAT positive.

Monica Agarwal comparison

	Sputum positive	CBNAAT positive	%	Sputum Negative	CBNAAT positive	%
My study	35	35	100	5042	32	0.0063
Monica study	21	21	100	123	33	0.26

Sensitivity and Specificity of CBNAAT

	Overall pulmonary TB		Sputum positive cases		Sputum negative cases	
Name of the study	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Dawan R *et.al.,*	40%	100%	100%	100%	32.3%	100
Theron *et.al.,*	78.7%	94.4%	94.7%	95%	46.8%	94.4%
Geleta *et.al.,*	65.5%	96.3%	95.2%	96.3%	48.6%	96.3%
Agarwal *et.al.,*	86.8%	93.1%	100%	90%	79.1%	99.6%
Sharma *et.al.,*	95.7%	99.6%	99.2%	99.6%	77.7%	99.6%
Sowjana.DS *et.al.,*	70.24%	100%	99.08%	100%	37.5%	100%
Boehme.CC *et.al.,*	92.2%	99.9%	98.2%	99.2%	72.5%	99.2%

DISCUSSION

Pulmonary tuberculosis is the leading cause for mortality and morbidity due to infectious disease in India inimmunocompromised and immunocompetent individuals India accounts for around one-fourth of the global tuberculosis cases (10). For long time sputum microscopy is the only rapid, simple, specific, cost effective, can be done in all levels of health care system with minimal facilities and training. The early specific diagnosis is essential step in reducing the mortality morbidity and in preventing spread of the disease in community.

WHO endorsed CBNAAT as diagnostic tool for pulmonary and extra pulmonary tuberculosis which has higher specificity and sensitivity when compared to sputum microscopy (11). India introduced CBNAAT under RNTCP for the diagnosis of Pulmonary and extra pulmonary TB With special reference to

all previously treated, PLHIV, Pediatric, close contacts of MDR TB.

This shows that significant numbers of symptomatic patients with smear negative status are misdiagnosed leading to progression and spread of the disease, challenging the control measures.

CONCLUSION

CBNAAT is a rapid and significantly useful in diagnosis of PTB when compared to sputum smear examination. Sensitivity and specificity is high compared to sputum smear examination and has significant effect on detecting undiagnosed presumptive cases. This will help in improving the TB control measures.

Detection of Rifampicin resistance one additional advantage for CBNAAT to screen for MDRTB and to decrease the spread MDRTB in community. The routine use of CBNAAT will improve detection drug sensitive and drug resistance TB and will have significant effect on TB control. CBNAAT Should use in special group of patient with presumptive PTB in sputum negative, all retreatment cases, PLHIV and Diabetes mellitus.

As Kanyakumari is educated cases we have very few new cases are detected. Because of discontinued therapy MDR Tb and XTR TB cases are detected.

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How to cite this article:

Gnanaguru, P, Murali R., and Subash Chandra Bose (2019) 'Evaluation of the Genexpert Mtb/Rif Assay for Rapid Diagnosis of Tuberculosis and Detection of Rifampin Resistance in Pulmonary and Extra Pulmonary Specimens', *International Journal of Current Advanced Research,* 08(02), pp. 17248-17250. DOI: http://dx.doi.org/10.24327/ijcar.2019.17250.3224

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