

A comparative study of AFB smear and GeneXpert MTB/RIF assay in pulmonary and extrapulmonary specimens and detection of rifampicin resistance in a tertiary care hospital

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Abstract

Tuberculosis is a major public health problem worldwide including India causing increased morbidity and mortality. There is a need for rapid identification for early diagnosis and treatment. In this study, GeneXpert MTB/RIF assay is used for rapid identification and was compared with AFB staining (by Zeihl Neelsen method). GeneXpert was used for *Mycobacterium tuberculosis* (MTB) complex identification and rifampicin detection from smear positive and smear negative patients. Out of 320 patients, 19 patients were acid-fast bacilli (AFB) smear positive and 67 patients detected for MTB by MTB/RIF assay. Rifampicin resistance (RR) was detected in 2 cases out of 62 positive cases by MTB/RIF assay. The test is considered more sensitive for AFB smear positive and showed high level of detection of MTB but less sensitive for AFB smear negative and showed low and medium level of detection of MTB. The GeneXpert is considered as an effective diagnostic tool for rapid diagnosis of MTB and RR in smear negative and positive specimens.

Keywords: *Mycobacterium tuberculosis*; GeneXpert MTB/RIF assay; rifampicin resistance; acid fast bacilli staining; pulmonary; extrapulmonary

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Introduction

In 2016, globally there were an estimated 10.4 million incident cases of tuberculosis (ranging from 8.8 million to 12.2 million), equivalent to 140 cases per 100,000 population. Most of the estimated number of cases in 2016 occurred in the WHO South-East Asia Region (45%). The five countries that stood out, as having the largest number of incident cases were India, Indonesia, China, the Philippines and Pakistan, which together accounted for 56% of the global total. Of these, China, India and Indonesia alone accounted for 45% of global cases. Nigeria and South Africa each accounted for 4% of the global total [1].

India stands highest, in burden of tuberculosis (TB). The World Health Organisation (WHO) TB

statistics for India for 2016, estimated 2.79 million cases for India. Incidence of multi-drug resistance tuberculosis (MDR TB) with rifampicin resistance is 11 per 100,000 population [2].

GeneXpert MTB/RIF assay (Cepheid, USA) is a rapid molecular test for diagnosis of TB recommended by WHO. It was initially recommended (in 2010) for diagnosis of pulmonary TB in adults. Since 2013, it has also been recommended for use in children and to diagnose specific forms of extrapulmonary TB (EPTB). The test has much better accuracy than sputum smear microscopy [1]. GeneXpert MB/RIF assay is currently used to detect *Mycobacterium tuberculosis* (MTB) complex DNA and its susceptibility to rifampicin (RMP) in a single reaction. It is based on seminested real-time PCR (rT-PCR) that targets the rpo-B gene hot spot region [3, 4]. Monoresistance to RMP is rare, however, >90% of RMP resistant isolates also exhibit resistance to isoniazid (INH). Therefore, the detection of RMP resistance may serve as surrogate marker of MDR *Mycobacterium tuberculosis* [5, 6]. The test is carried out within 2 hours in a disposable cartridge [7].

Early diagnosis is helpful for early patient management and successful patient outcomes. False-negative results and misdiagnosis of TB suspects are common in developing nations, as most TB control programmes use Ziehl-Neelsen (ZN) smear microscopy, which has poor sensitivity and multiple visits are required which leads to higher default. Mycobacterial culture, although considered as the gold standard but is slow and usually takes 2-6 weeks time to yield a final result and requires proper infrastructure and technical expertise [8-10]. Diagnosis of EPTB is difficult to establish due to the low number of bacteria in clinical specimens. Rapid and accurate diagnosis of pulmonary and EPTB is still a great challenge [11].

In the present study, aim is to study pulmonary and extrapulmonary specimens by AFB staining (Ziehl Neelsen method) and molecular method of detection by cartridge based nucleic acid amplification test (CB-NAAT) or GeneXpert MTB/RIF assay (Cepheid, USA) in patients attending to a tertiary care hospital.

AIM: 1. To detect MTB by GeneXpert MTB/RIF in inpatient (IP) and outpatient (OP) and to find out the incidence rate, 2. To find out predominant gender and age group for MTB in IP and OP, 3. To compare pulmonary and extrapulmonary AFB smear report with GeneXpert RIF/MTB assay, along with detection of rifampicin resistance.

Materials and method

The study was conducted in the Department of Microbiology at Krishna Institute of Medical Sciences Ltd., Secunderabad, Telangana. The study duration was one year (1 August 2016- 31 July 2017). It is a laboratory based prospective study. A total of 320 various clinical specimens of tuberculosis suspected patients from inpatients and outpatients were studied. A total of 198 pulmonary specimens (bronchial wash, pleural fluid, sputum, ET secretion and tracheal secretion) and a total of 122 extrapulmonary specimens (tissue biopsy, lymph nodes, ultrasound guided aspiration, FNAC, pus, CSF, drain fluid, ascitic fluid, urine) were included in the study.

Inclusion criteria: All pulmonary and extrapulmonary specimens from IP and OP were received with request of two tests: 1. AFB smear examination by Ziehl Neelsen method and 2. GeneXpert MTB/RIF assay, which were included in this study.

Exclusion criteria: Those specimens which had only one method, either GeneXpert or AFB smear by Ziehl Neelsen staining method were excluded from the study.

Ziehl Neelsen staining method for detection of MTB

The smear preparation was done in biosafety cabinet level 2B. For sputum specimens, a direct smear and a concentration method smear (by Petroff's method) were prepared and heat fixed. Acid fast bacilli (AFB) staining was done by Ziehl Neelsen staining method and then visualised under 40x and 100x light microscope [12, 13]. Reporting of sputum is done as per RNTCP guidelines [13, 14].

GeneXpert MTB/RIF assay (Figure 1a,b)

It detects MTB DNA in under 2hrs and detects mutations that cause resistance to rifampicin (Figure 1). Sample reagent was added at a 2:1 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 was transferred to the test cartridge. The last result was used for the analysis [15].

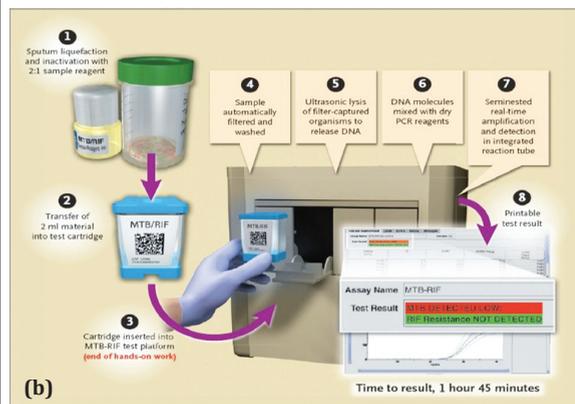
A total of 320 specimens data were collected in excel format and analysed by using SPSS 20.

Results

Out of 320 clinical samples, 196 IP and 124 OP specimens were collected. Out of 196 IP, 25 MTB



(a)



(b)

Figure 1: (a) GeneXpert MTB/RIF assay machine, (b) Stepwise procedure of GeneXpert.

detected by GeneXpert and Out of 124 OP, 42 MTB were detected by GeneXpert MTB/RIF assay (Table 1).

Table 1: *Mycobacterium tuberculosis* detected by GeneXpert MTB/RIF assay in inpatient and outpatient specimens.

	IP; n=196 (61.25%)	OP; n=124 (38.75%)
<i>M. tuberculosis</i> not detected	171 (87.24%)	82 (66.13%)
<i>M. tuberculosis</i> detected	25 (12.76%)	42 (33.87%)
Low	19	23
Medium	2	12
High	4	7

Among 196 IP patients, male was predominant gender (n=133) and among 124 OP patients, female was predominant gender (n=70) (Table 2).

Table 2: *Mycobacterium tuberculosis* detected by GeneXpert MTB/RIF assay in Males and Females.

	IP (n=196)	OP (n=124)	Total (n=320)
Male	133 (67.86%)	54 (43.55%)	187 (58.44%)
Female	63 (32.14%)	70 (56.45%)	133 (41.56%)

Among different age groups, 41-60years was most commonly affected age group followed by 21-40 years (Table 3).

Table 3: *Mycobacterium tuberculosis* detected by GeneXpert MTB/RIF assay age group wise.

Age group	IP (n= 196)			OP (n= 124)			Total (n=320)
	Age	M	F	Age	M	F	
<20	10	5	5	7	2	5	17 (5.31%)
21-40	46	30	16	54	11	43	100 (31.25%)
41-60	74	56	18	36	20	16	110 (34.38%)
61-80	59	38	21	22	15	7	81 (25.31%)
>81	7	4	3	5	4	1	12 (3.75%)

Abbreviations: M: Male; F: Female.

Among all IP patients, 8 were AFB smear positive and 188 were AFB smear negative. Among all OP patients, 11 were AFB smear positive and 113 were AFB smear negative. All smear positive and negative patients were subjected to GeneXpert MTB/ RIF assay (Table 4).

Table 4: *Mycobacterium tuberculosis* detected by AFB staining by Zeihl Neelsen method.

	AFB smear positive	AFB smear negative
IP	8 (4.08%)	188 (95.92%)
OP	11 (8.87%)	113 (91.13%)
Total	19 (5.94%)	301 (94.06%)

Among 8 smear positive IP patients, 7 patients were GeneXpert positive and one was negative. Among 188 smear negative IP patients, 18 patients were GeneXpert positive and 170 were negative. Among 11 smear positive OP patients, 11 patients were GeneXpert positive. Among 113 smear negative IP patients, 31 patients were GeneXpert positive and 82 were negative (Table 5).

Table 5: GeneXpert MTB/RIF assay result among AFB smear positive and AFB smear negative patients.

	<i>IP patients</i>		<i>OP patients</i>	
	<i>AFB smear positive</i>	<i>AFB smear negative</i>	<i>AFB smear positive</i>	<i>AFB smear negative</i>
GeneXpert not detected	1 (0.51%)	170 (86.74%)	0	82 (66.13%)
GeneXpert detected	7 (3.57%)	18 (9.18%)	11 (8.87%)	31 (25%)
Low	2	17	4	19
Medium	1	1	2	10
High	4	0	5	2

Abbreviations: IP: inpatient; OP: outpatient; AFB: acid- vast bacilli.

Among various sample, 198 pulmonary specimens and 122 extrapulmonary specimens. Out of 198 pulmonary samples, 38 MTB were detected and

among 122 extrapulmonary, 29 MTB were detected by GeneXpert MTB/RIF assay (Table 6).

Table 6: AFB smear report, GeneXpert report along with rifampicin resistance in pulmonary and extrapulmonary specimens.

<i>S. No</i>	<i>Pulmonary samples (n=198; 61.88%)</i>	<i>Number (%)</i>	<i>AFB positive report</i>	<i>GeneXpert positive report</i>				<i>Rifampicin resistance detected</i>
				<i>No.</i>	<i>L</i>	<i>M</i>	<i>H</i>	
1	Bronchial wash	100(50.50)	7	17	9	4	4	Not detected
2	Pleural fluid	62(31.31)	2	7	6	-	1	Not detected
3	Sputum	31(15.66)	7	11	4	2	5	Not detected
4	ET secretion & Tracheal secretion	5(2.53)	Nil	Nil	-	-	-	Not detected
Total		198	16(8.08)	35(17.68)	19	6	10	
<i>S.No</i>	<i>Extra pulmonary samples (n=122; 38.12%)</i>	<i>Number (%)</i>	<i>AFB positive report</i>	<i>GeneXpert positive report</i>				<i>Rifampicin resistance detected</i>
				<i>No.</i>	<i>L</i>	<i>M</i>	<i>H</i>	
1	Tissue biopsy	48(39.34%)	Nil	10	8	2	-	1
2	Lymph node	16(13.11)	1	5	4	1	-	Not detected
3	Ultrasound guided aspiration	14(11.48)	Nil	5	3	2	-	Not detected
4	FNAC	13(10.66)	Nil	4	3	1	-	Not detected
5	Pus	12(9.84)	1	6	5	2	-	1
6	CSF	09(7.38)	Nil	Nil	-	-	-	Not detected
7	Drain fluid	05(4.10)	1	1	1	-	-	Not detected
8	Ascitic fluid	03(2.45)	Nil	Nil	-	-	-	Not detected
9	Urine	02(1.64)	Nil	1	1	-	-	Not detected
Total		122	3(2.45)	32(26.23)	25	8	-	

In this study, it was observed that patients can be categorized according to their AFB staining report and GeneXpert MTB/RIF assay report in both inpatients and outpatients (Table 7).

Table 7: Observation of AFB staining report & GeneXpert MTB/RIF assay report in both inpatients and outpatients.

	Pulmonary specimens		Extrapulmonary specimens		Total
	IP	OP	IP	OP	
AFB smear + GeneXpert +	6	9	2	2	19
AFB smear - GeneXpert +	18	10	5	21	54
AFB smear + GeneXpert -	1	0	0	0	01
AFB smear - GeneXpert -	115	39	49	43	246
Total	140	58	56	66	320

Abbreviations: IP: inpatient; OP: outpatient; AFB: acid- vast bacilli.

Discussion

In the prospective study, the diagnostic yield of GeneXpert to detect MTB in pulmonary and extrapulmonary specimens was evaluated and compared with AFB smear staining by Zeihl Neelsen staining. GeneXpert is a simple bench top point of care diagnostic assay that can be performed with minimal training. The results are available within 2 hours, much earlier than the culture which usually takes days to come positive [16-18].

Steingart et al. showed a systematic review of 27 studies, in which GeneXpert assay of respiratory specimens had a pooled sensitivity of 89% (95% CI: 85%–92%) and specificity of 99% (95% CI: 98%–99%) in the diagnosis of pulmonary TB [19]. Zeka et al. [20] showed that due to low number of bacilli in extrapulmonary samples, they are not detected by AFB staining frequently. They are detected by GeneXpert MTB/RIF assay, which were the limits of detection of the test, as comparable to the present study as shown in Table 6.

Out of 320 patients screened for AFB smear and GeneXpert MTB/RIF assay, inpatients were 196 (61.25%) and outpatients 124 (38.75%). Incidence rate for MTB detected by GeneXpert was higher in outpatients i.e. 42 (33.87%) followed by 25 inpatients (12.76%). Gender predominance seen in male (n=187; 58.44%) followed by female (n=133;

41.56%). The most common age group affected was 41-60 years (n=110; 34.38%) followed by 21-40 years (n=100; 31.25%), 61-80 years (n=81; 25.31%), <20years (n=17; 5.31%) and <80years (n=12; 3.75%). Out of 198 (61.88%) pulmonary specimens [bronchial wash(n=100; 50.50%), pleural fluid (n= 62; 31.31%), sputum (n=31; 15.66%), ET secretion and tracheal secretion (n=05; 2.53%)] were detected by MTB/RIF assay followed by 122 (38.12%) extrapulmonary specimens [tissue biopsy (n=48; 39.34%), lymph nodes (n=16; 13.11%), ultrasound guided aspiration (n=14; 11.48%), FNAC (n=13; 10.66%), pus(n= 12; 9.84%), CSF (n=09; 7.38%), drain fluid (n=05; 4.10%), ascitic fluid (n=03; 2.45%), urine(n=02; 1.64%)].

Out of 198 pulmonary specimens, 16 (8.08%) were AFB smear positive and 35 (17.68%) were GeneXpert positive. One sample was found to be AFB staining positive and GeneXpert negative. The probable reason for false negative result would be PCR inhibitors present in the assay.

Out of 122 extrapulmonary specimens, 3 (2.45%) were AFB smear positive and 32 (26.23%) were GeneXpert positive. High level detection of MTB is observed in pulmonary specimens [sputum (5) followed by bronchial wash (4) and pleural fluid (1)]. In extrapulmonary specimens, low level detection of MTB is observed. High level detection was observed in AFB smears positive cases. Rifampicin resistance was seen in only two cases of extrapulmonary specimens (one in tissue biopsy and one in pus). However, GeneXpert does not eliminate the need of conventional microscopy, culture and anti-tubercular drug sensitivity that are required to monitor the progression of treatment and to detect resistance to drugs other than Rifampicin [21].

Conclusion

The GeneXpert MTB/RIF assay is a rapid and easy-to-perform fully automated Nucleic acid amplification test, which is extremely helpful in early diagnosis and to initiate the treatment of tuberculosis in extrapulmonary and pulmonary tuberculosis. It has a high capability to detect MTB complex DNA in AFB microscopy negative samples of pulmonary and extrapulmonary origin. The assay correctly detects the information from the rpoB hot spot region regarding rifampicin resistance. The early diagnosis by MTB/RIF assay and to initiate correct regimen with regular follow up would be helpful in management of tuberculosis patients.

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Conflict of interest

The authors declare no conflict of interest.

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