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GeneXpert MTB/RIF as a Rapid Diagnostic tool for Diagnosis of Pulmonary, Extra-pulmonary Tuberculosis and Rifampicin Resistance in the Egyptian Population

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAMA designed the study, shared in the laboratory work, managed the literature searches, managed the analyses of the results and wrote the first draft of the manuscript. Author RMH wrote the protocol, participated in the designing of the study shared in the laboratory work and reviewed of the manuscript. Author MKS participated in the designing of the study, shared in the laboratory work, performed the statistical analysis and reviewed of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Rapid and accurate detection of *Mycobacterium tuberculosis* complex (MTBC) with early detection of drug-resistance is mandatory for the effective control of TB.
Aim: An evaluation of the performance of the GeneXpert MTB/RIF assay in the diagnosis of pulmonary and extra-pulmonary TB and Rifampicin (RIF) resistance.
Materials and Methods: 420 patients with clinical or radiological suspicion of Tuberculosis were included in this study. GeneXpert MTB/RIF (Cepheid, Sunnyvale, USA) was used for diagnosis of pulmonary, extra-pulmonary tuberculosis and Rifampicin-resistance. Comparison of our results was done with the results of acid-fast bacilli (AFB) smear microscopy and the culture method (as a

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standard method). Rifampicin resistance results were compared to drug susceptibility testing (DST) as a gold standard method. Sequencing of the *rpoB* gene was done for the Rifampicin-resistant isolates using genetic analyser ABI prism 3130 XL (Applied Biosystems, USA).

Results: TB was diagnosed in 23.7% of patients suspected to have pulmonary TB and in 12.5% of patients suspected of having extra-pulmonary TB. The GeneXpert MTB/RIF detected MTB with an overall sensitivity of 88.7% (63/71), specificity of 97.8% (224/229), PPV of 92.6% and NPV of 96.5%. There was a highly statistically significant difference between GeneXpert MTB/RIF and AFB smear microscopy, in pulmonary and extra-pulmonary cases. RIF resistance was identified by DST in 2.3% (2/86) TB culture positive specimens; both of which were multidrug-resistant (MDR). The GeneXpert MTB/RIF assay showed RIF resistance in all specimens identified as RIF resistant by DST in addition to one specimen identified as RIF susceptible by DST. The results of gene sequencing were in concordance with that obtained from GeneXpert MTB/RIF.

Conclusions: Our findings inforce the use of GeneXpert MTB/RIF as a prompt rapid diagnostic tool for early diagnosis of pulmonary TB especially in smear-negative and extra-pulmonary tuberculosis with the early detection of Rifampicin resistance.

Keywords: Tuberculosis; GeneXpert MTB/RIF; Rifampicin resistance and Gene sequencing.

ABBREVIATIONS

TB : Tuberculosis
MDR : Multidrug-resistant
INH : Isoniazid
RIF : Rifampicin
MTB : Mycobacterial tuberculosis complex
DST : Drug susceptibility testing
NAAT : Nucleic Acid Amplification Test
rpoB : RNA polymerase beta
RRDR : RIF resistance-determining region
AFB : Acid-fast bacilli
CLSI : Clinical and Laboratory Standards Institute
LJ : Löwenstein-Jensen
EMB : Ethambutol
BZA : Pyrazinamide
MGIT : Mycobacterial growth indicator tube

1. INTRODUCTION

Tuberculosis (TB) is still a disease with a high fatality rate worldwide. Although incidence rates of TB are in decline, it remains a leading cause of death. Multidrug-resistant (MDR)-TB means the presence of resistance to Isoniazid (INH) and Rifampicin (RIF) simultaneously and it is a major public health problem [1].

WHO reported that in the year 2015, only 59% of the new tuberculosis cases could be diagnosed (6 from 10.5 millions). Simultaneously in the same year, only 125000 rifampicin-resistant cases could be identified from 580,000 cases (20%). These reports indicated that there is a wide gap in the detection of *Mycobacterial tuberculosis* complex (MTBC) and rifampicin resistance that is caused most probably by the lack of easily accessible, sensitive and adequately rapid method for diagnosis of TB [2].

Rapid and accurate detection of MTBC with early detection of drug-resistance is mandatory for the effective control of TB. Although, smear microscopy is a rapid, simple and inexpensive screening method, however, its effectiveness as a diagnostic tool is limited by a very poor sensitivity. The conventional culture method for TB identification and standard direct sensitivity test (DST) are still regarded as the gold standard for diagnosis of TB and detection of drug resistance until now, however, they need a long duration of more than 6 weeks [3].

Drug resistance surveillance data showed that 5% of TB cases were estimated to have MDR-TB in 2014 [4]. Detection of Rifampicin resistance is regarded as a valid method for detection of MDR-TB as a large proportion of Rifampicin-resistant strains also have a noticeable resistance to isoniazid (INH) [5].

The GeneXpert MTB/RIF (Cepheid, Sunnyvale, USA) is a fully automated real time semi-nested PCR system, based on molecular beacon technology. It is a cartridge-based Nucleic Acid Amplification Test (NAAT) used for detection of both MTBC and RIF resistance within 2 hours. It allows for DNA isolation, concentration and amplification of MTB. Three primers are used for amplification of the MTBC-specific sequence of the RNA polymerase beta (*rpoB)* gene, and five molecular probes for detection of mutations within the gene's RIF resistance-determining region (RRDR) [6-7]. Both raw sputum samples and concentrated sediments can be used for the assay of MTBC and RIF resistance. It has been recommended by the WHO in 2010 and approved by the FDA in 2013 as an initial diagnostic tool for individuals suspected of

multidrug-resistant tuberculosis or HIV-associated tuberculosis [8].

Our study aimed to evaluate the GeneXpert MTB/RIF assay as a rapid diagnostic tool for the diagnosis of pulmonary and extra-pulmonary TB in addition to rapid detection of RIF resistance, compared to acid-fast bacilli (AFB) smear microscopy and the culture method (as a standard method) and with DST as gold standard methods, in Egyptian population.

2. MATERIALS AND METHODS

2.1 Study Design

This study was carried out on 420 patients with clinical/radiological suspicion of Tuberculosis. The study was conducted from Menofia Chest Hospital & Abbasia Chest Hospital – Cairo, Ministry of Health, Egypt, from May 2015 till December 2016, on 300 patients suspected of having pulmonary TB and 120 suspected of having extra -pulmonary TB. Patients were either without treatment [253 (84.3%) of pulmonary and 106 (88.3%) of extra-pulmonary], or were on anti-TB treatment for not more than two weeks [5 (1.7%) pulmonary and 2 (1.7%) extra-pulmonary]. Patients who were on anti-TB treatment for more than 2 weeks were excluded from the study**.** The study protocol was approved by the local ethical committee number of protocol 9H/18.

2.2 Sample Collection

A total of 300 pulmonary samples: sputum and Broncho-alveolar lavage (BAL) and 120 extra-pulmonary samples (pleural fluid, ascetic fluid, urine and pus) were collected in plain universal 30 ml clear plastic container with white cap. All samples were collected from the hospital laboratory. No extra samples were withdrawn for the sake of the research. Samples were divided into three parts, one for AFB smear microscopy, second for culture and third for the GeneXpert MTB/RIF assay. The Rifampicin resistant strains are stored at -80°C for further processing for detection of gene mutation by sequencing.

2.3 Smear Microscopy

Ziehl-Neelsen stain was used for staining of all specimens. The grading of AFB positivity was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines to one of the four categories (1+, 2+, 3+, 4+). Three successive mornings sputum samples were examined from each pulmonary TB suspected patient [9-10].

2.4 Culture

Specimens were decontaminated using N-Acetyl-L-Cysteine-Sodium Hydroxide (NALC NaOH) (MycoPrep, Becton Dickinson, USA) , then specimens were inoculated in mycobacterium growth indicator tube (MGIT) for liquid culture on the BACTEC 960 instrument (Becton Dickinson, USA) and on the slope of Löwenstein-Jensen (LJ) solid medium (Heipha Diagnostika Biotest, Germany) [11]. Solid and liquid cultures were considered negative if no growth of any Mycobacteria could be detected after 6 weeks (42 days) of incubation.

2.5 Identification of Mycobacteria

Positive culture were identified as MTB from growth on liquid culture using immune-chrommatographic based test (Standard Diagnostics SD TB Ag MPT 64 Rapid) (SD, South Korea) and from growth on solid media by their slow growth rate, colony morphology, catalase test and niacin test. Any sample that was detected as nontuberculous mycobacterium (NTM) by culture method was excluded from the study [12].

2.6 Drug Susceptibility Test (DST)

Susceptibility test was performed to first-line anti-tuberculous drugs which are Rifampicin 1.0 µg/mL (RIF), Isoniazid 0.1 µg/mL (INH), Ethambutol 5.0 µg/mL (EMB), Pyrazinamide 100 µg/mL (PZA) using the automated Myco-bacterial growth indicator tube (MGIT) BACTEC 960 system (Becton Dickinson, USA) using a BACTEC MGIT SIRE kit (Becton Dickinson, USA) according to the instructions provided by the manufacturer. The results of DST were considered as the gold standard for drug susceptibility results [13].

2.7GeneXpert MTB/RIF Procedure

The GeneXpert MTB/RIF (Cepheid, Sunnyvale, USA) was used for detection of both MTBC and RIF resistance. In this assay molecular beacon technology is used for detection of DNA sequences which amplified in a hemi-nested real time PCR assay [14]. Decontaminated specimen was used in an amount of 0.5 mL, to which

sample reagent was added in a ratio 2:1. The closed tube was incubated at room temperature for 15 minutes and manually agitated twice during the incubation period. The sample was inactivated with a special reagent supplied in the detection kit; then two mL of that mixture is transferred to the GeneXpert test cartridge which is inserted into the GeneXpert instrument after paring it with the patient data applied to the instrument. Specimen was considered positive for M. tuberculosis when two probes give positive signals, minimally and that at a cycle threshold (CT) of ≤38 cycles. The standard result obtained from the instrument gives the signal indicating the presence or absence of *M. tuberculosis* and the presence or absence of Rifampicin resistance. *Bacillus globigii* is used as an internal control and is considered positive when its single probe produces a CT of ≤38 cycles. Assays that are negative for *M. tuberculosis* and for *B. globigii* are reported as invalid assays and were excluded from the study [15] (Fig. 1A-1B).

2.8 Sequencing of *rpoB* Gene

This was performed on the Rifampicin resistant isolates either by GeneXpert system or DST. DNA was isolated using Genolyse buffer (Hain life sciences) according to the manufacturer's instructions. Amplification of *rpoB* gene was done using primer sequence (Table 1) as previously described, yielding 280 bp products. PCR was carried out in 100 µL containing 10 mM Tris (pH 8.0), 25 mM MgCl2, 10 mM KCl, 10 µM dNTPs, 2U Taq polymerase, and 5 ng of *rpoB* primers. PCR cycles were adjusted as following one cycle at 94°C for 1 min, followed by 35 cycles denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and a final cycle at 72°C for 5 min. Sequencing of 81-bp rpoB gene was done directly using the genetic analyser ABI prism 3130 XL (Applied Biosystems, USA). The results of sequencing were compared to standard M. tuberculosis H37Rv strain, GenBank accession reference numbers 888164 [16-17].

2.9 Statistical Methods

Data were analysed using IBM SPSS Statistics, version 23.0 (IBM Corp., Armonk, NY). Sensitivity, specificity and negative and positive predictive values were also calculated for evaluating the performance of the GeneXpert assay in the detection of MTB and detection of RIF resistance.

Table 1. Primers used for the amplification of *rpoB* gene

Gene	Primer sequence (5´-3´)	Amplicon size (pb)	Reference
rpoB-F	AGCGGATGACCACCCAGGAC	280	[17]
rpoB-R	TCAGGGGTTTCGATCGGGCA		

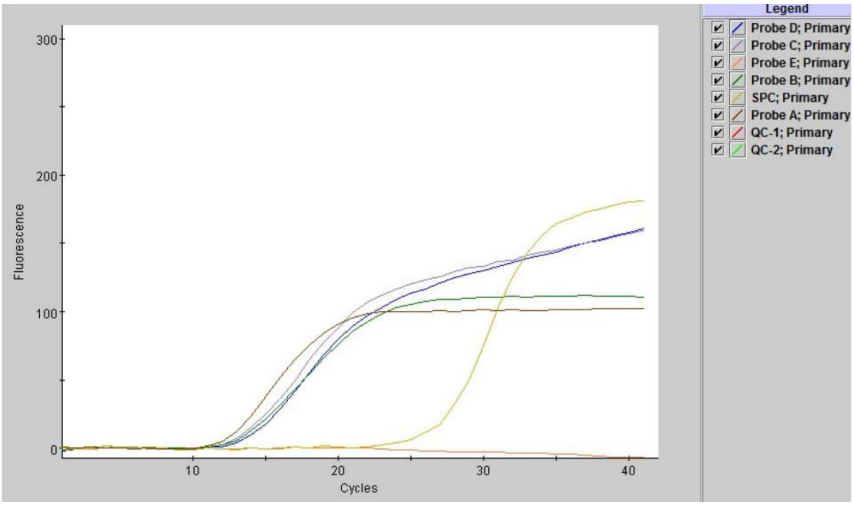


Fig. 1(A). Curves obtained from GeneXpert cycle showing a positive result for (MTBC) with positive Rifampicin resistance (Rifampicin resistant)

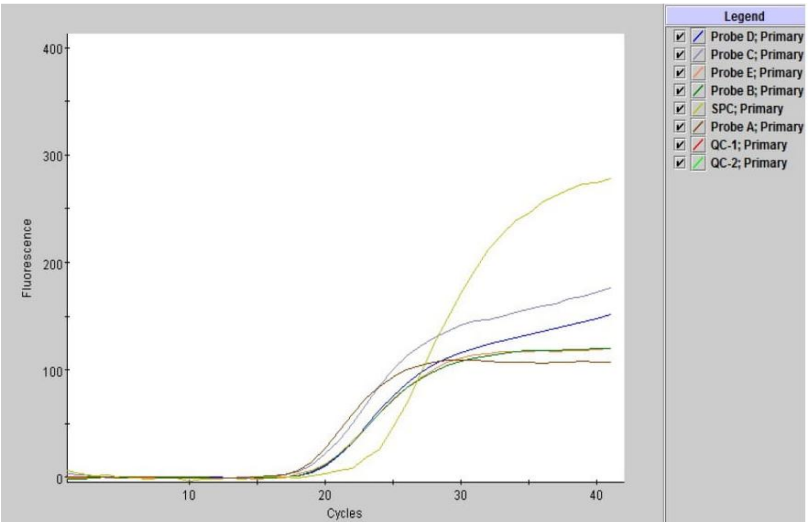


Fig. 1(B). Curves obtained from GeneXpert cycle showing a positive result for (MTC) with a negative result for Rifampicin resistance (rifampicin susceptible)

3. RESULTS

Four hundred and twenty specimens were collected from patients suspected of having TB. From 300 patients having pulmonary symptoms, 71 (23.7%) specimens were TB positive; while 229 (76.3%) specimens were TB negative based on culture results. While from 120 patients with extra pulmonary symptoms, 15 (12.5%) were culture positive for TB and 105 (87.5%) were culture negative (Table 2). The source of specimens for pulmonary cases were mainly sputum (96%) while the main source of specimen for extra pulmonary cases were pleural fluid (45%) and urine (35%) (Fig. 2). The mean age of the pulmonary TB positive patients was (39.9 ± 15.3) and (40.6 ± 17.1) in extra pulmonary TB cases, with male gender predominate in both groups; 70.4% and 73.3% in pulmonary and extra-pulmonary TB groups, respectively. Rural residency was more common in pulmonary TB (91.5%), and nearly equal in extra-pulmonary TB to urban residence (53.3%). Ten over 420 of studied patients were HIV infected (2.4%) and all 10 HIV cases were TB positive. Cough was the most presenting symptom in pulmonary TB (97.2%) with abnormal chest X-ray in 94.4%, while night sweat and weight loss were the most presenting symptoms in extra-pulmonary TB cases (40% and 53.3%, respectively). Most of our patients have no history of prior treatment (Table 3).

The performance of the GeneXpert MTB/RIF assay for the detection of MTBC in pulmonary TB was evaluated against culture as the gold standard for TB diagnosis. GeneXpert MTB/RIF detected MTB with a total sensitivity of 88.7% (63/71), 95.2% (40/42) among smear-positive and 79.3% (23/29) among smear-negative sputa. The specificity of GeneXpert MTB/RIF was 97.8% (224/229) total; 77.8% (14/18) among smear-positive and 99.5% (210/211) among smear-negative (Table 4). Twenty two out of twenty three showing GeneXpert MTB/RIF positive patients while their ZN smears were negative, were found to have findings in their chest X- ray. Eight specimens diagnosed as negative by GeneXpert MTB/RIF, while their culture result was positive, their ZN smear results demonstrated Six were negative and two were smear positive (+1). Five specimens were collected from patients receiving anti-TB treatment at the time of testing, all show GeneXpert-positive and culture-negative, (false positive by Genexpert assay) including all four smear-positives.

AFB smear microscopy demonstrated a sensitivity of 59.2% (42/71), specificity of 92.1% (211/229), PPV 70% (42/60) and NPV 87.9% (211/240), respectively for the detection of mycobacteria in pulmonary TB suspected patients with high statistically. Significant difference between GeneXpert MTB/RIF and AFB smear microscopy eighteen specimens were identified as a smear-positive while their culture revealed negative results, 14 over those 18 were correctly diagnosed by GeneXpert MTB/RIF assay as negative. Also from the 29

identified as a smear negative while their culture results were positive, 23 were correctly detected as positive by GeneXpert MTB/RIF assay (Table 4).

The performance of the GeneXpert MTB/RIF assay for the diagnosis of extra pulmonary TB demonstrated a sensitivity of 80.0% (12/15), specificity of 100% (105/105), PPV 100% (12/12) and NPV 97.2% (105/108), respectively. AFB smear microscopy demonstrated a sensitivity of 26.7% (4/15), specificity of 100% (105/105), PPV 100% (4/4) and NPV 90.5% (105/116), respectively with high statistically significant difference. Eleven from extra pulmonary specimens were identified as negative by ZN smear-negative while their culture revealed positive results, 8/11 that microscopy failed to detect, were correctly detected by GeneXpert MTB/RIF. The 2 smear-positive and culture-negative specimens, both were correctly excluded by GeneXpert MTB/RIF (Table 5).

A total of 86 positive TB specimens (71 from pulmonary and 15 from extra pulmonary) were tested for resistance to the 4 first-line anti-TB agents (RIF, INH, PZA and EMB) by DST and revealed that 12 out of 86 (14.0%) were resistant to at least one of first line anti-tuberculosis agents, 10 out of 71 (14.1%) isolates from pulmonary cases and 2 out of 15 (13.3%) from extra pulmonary cases (Table 6).

RIF resistance was identified by DST in 2 (2.3%) of the 86 culture-positive specimens; both RIF resistant strains demonstrated resistant to INH (MDR) and the other 2 drugs of the first-line anti-TB agents tested. The GeneXpert MTB/RIF assay showed RIF resistance in three specimens the (two specimens which were identified by DST, and also in 1 specimen that DST identified it as RIF susceptible). All other 83 specimens that GeneXpert MTB/RIF detected as RIF susceptible were also identified as RIF sensitive by DST. GeneXpert MTB/RIF demonstrated sensitivity and specificity for the detection of RIF resistance 100% (2/2) and 98.8% (83/84), respectively, using DST as the gold standard (Table 7).

The sequencing results of the RIF resistant detected by GeneXpert revealed that for the two specimens with concordant results of GeneXpert and DST , RIF resistance were confirmed by detection of a point mutation in codon 531 (TCG/ TTG) (Ser/Leu) in both isolates. For the one specimen with discrepant GeneXpert and DST results, sequencing revealed that strain have a point mutation in *rpoB* gene in codon 511 (CTG/CCG) (Leu/ Pro) which reported to be associated with low-level RIF resistance but clinically significant level. Incorporating the results of sequencing, GeneXpert MTB/RIF specificity and PPV increased to 100 (83/83) and 100 (3/3), respectively (Table 7, Fig. 3A-B).

The highest mono-resistant detected by phenotypic DST was for INH as 8/86 (9.3%) PZA-mono-resistant and 2/86 (2.3%) of specimens were MDR; both of which were resistant to RIF, INH, PZA and EMB (Table 8).

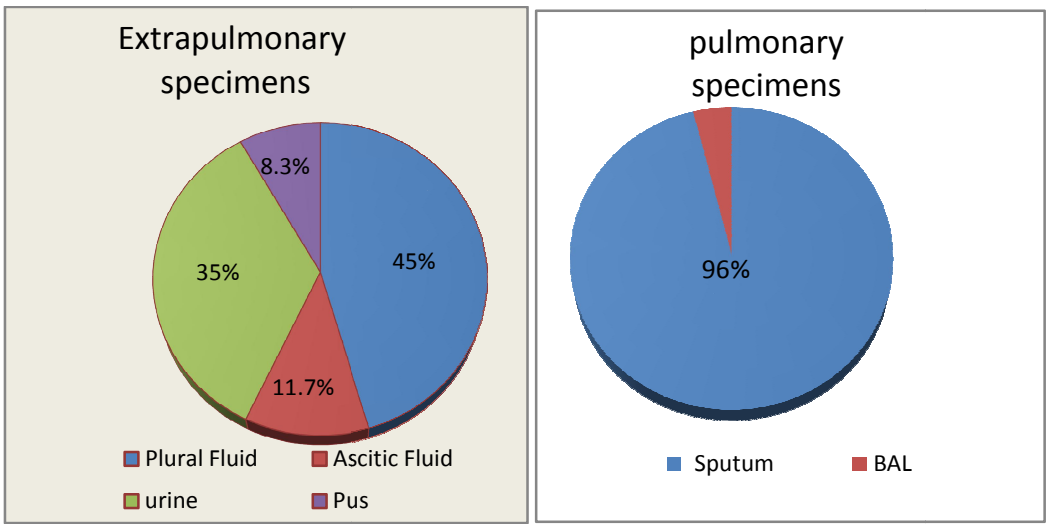


Fig. 2. Distribution of pulmonary and extra-pulmonary specimens

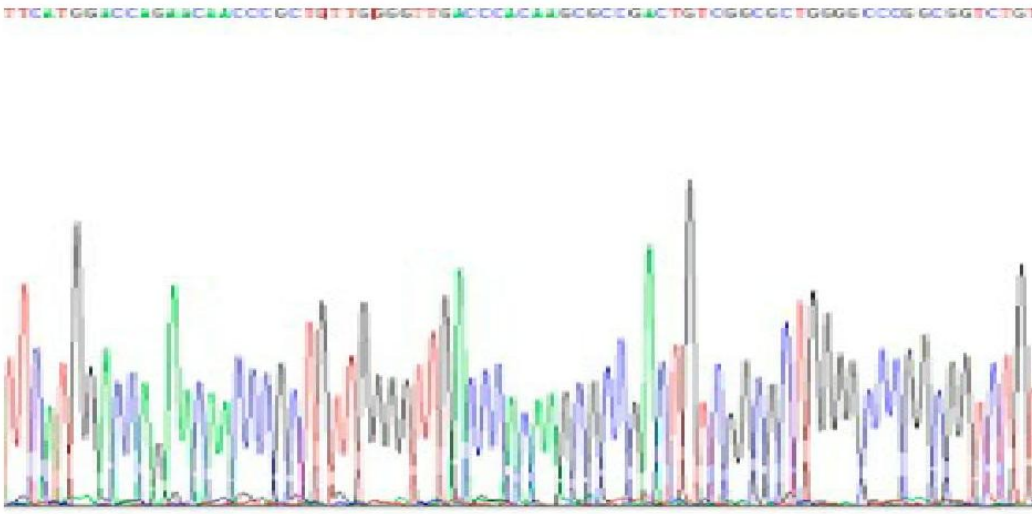
Table 2. Frequency of Tuberculosis (culture positivity) among the studied specimens

Specimen type	Total number	TB positive	TB negative
Pulmonary	300	71(23.7)	229 (76.3)
Extra Pulmonary	120	15 (12.5)	105 (87.5)

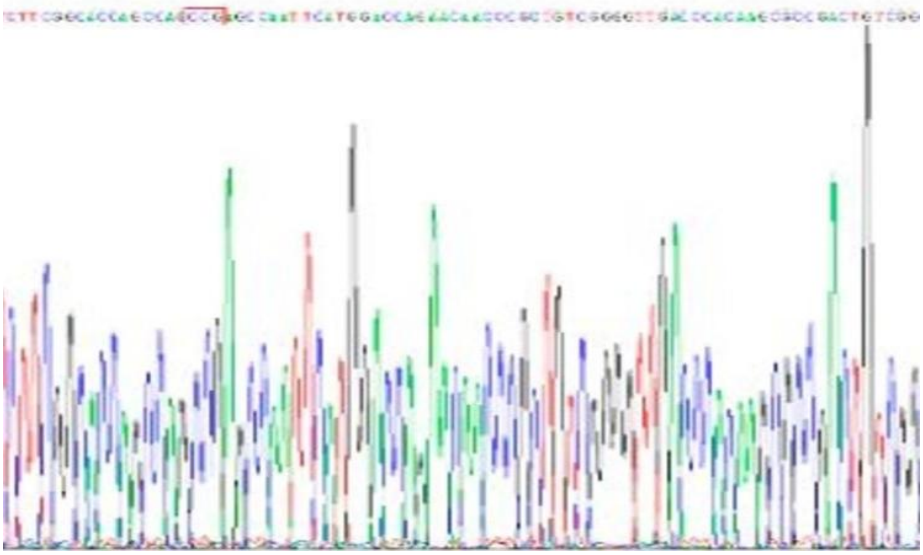
Table 3. Demographic data of studied population

Characteristic	Patients suspected of pulmonary TB			Patients suspected of extra - pulmonary TB		
	NEG 229	POS 71	total 300	NEG 105	POS 15	Total 120
Age (mean ± SD)	41.5±16.1	39.9 ±15.3	40.7±15.7	42.4±17.5	40.6±17.1	41.5±17.3
Male sex No (%)	137 (59.8)	50 (70.4)	187(62.3)	65 (61.9)	11 (73.3)	76 (63.3)
Rural area No (%)	182 (79.5)	65(91.5)	247 (82.3)	59(56.2)	8 (53.3)	67(55.8)
HIV infected No (%)	0 (0)	8 (11.3)	8 (2.7)	0 (0)	2 (13.3)	2 (1.7)
Presenting symptoms No (%)						
Cough	175 (76.4)	69 (97.2)	244 (81.3)	0 (0)	5 (33.3)	5 (4.2)
Fever	24 (10.5)	25 (35.2)	49 (16.3)	3 (2.9)	3 (20)	6 (5)
Weight loss	174 (76.0)	61 (85.9)	235 (78.3)	53 (50.5)	8 (53.3)	61(50.8)
Night sweats	92 (40.2)	43 (60.6)	135 (45)	47(44.8)	6 (40)	53 (44.2)
Abnormal CXR	27 (11.8)	67 (94.4)	94 (31.3)	0 (0)	3 (20)	3 (2.5)
Source of specimen No (%)						
Sputum	220 (96.1)	68 (95.8)	288 (96.0)	0 (0)	0 (0)	0 (0)
BAL	9 (3.9)	3 (4.2)	12 (4)	0 (0)	0 (0)	0 (0)
Pleural fluid	0 (0)	0 (0)	0 (0)	46 (43.8)	8 (53.3)	54 (45)
Ascitic fluid	0 (0)	0 (0)	0 (0)	12(11.4)	2 (13.3)	14 (11.7)
Urine	0 (0)	0 (0)	0 (0)	38(36.2)	4 (26.7)	42(35)
Pus	0 (0)	0 (0)	0 (0)	9 (8.6)	1 (6.7)	10 (8.3)
TB treatment history No (%)						
Current treatment	0 (0)	5 (7.0)	5 (1.7)	0 (0)	2 (13.3)	2 (1.7)
Prior treatment	13 (5.7)	29 (40.8)	42 (14)	5 (4.8)	7 (46.7)	12 (10)
No history treatment	216 (94.3)	37 (52.1)	253 (84.3)	100 (95.2)	6 (40)	106 (88.3)

TB: tuberculosis CXR Chest X ray BAL: broncho-alveoar lavage



(3A): Codon 531 (TCG/TTG) mutation site is marked by red lines



(3B): Codon 511(CTG/CCG) mutation site is marked by red lines

Fig. (3A&B). Mutations identified by sequanceing analysis of *rpo B* gene

4. DISCUSSION

Tuberculosis (TB) is a major health problem worldwide. In our study TB was diagnosed in 71/300 (23.7%) in pulmonary suspected cases while in extra pulmonary suspected cases 15/120 (12.5%) were TB positive. This is in agreement with the results of many Egyptian studies on TB cases [18-19]. So, Egypt is still considered as one of the high TB burden countries. For several years smear microscopy and conventional culture techniques have been the main stone for diagnosis of TB. Smear microscopy has poor sensitivity while conventional culture techniques needs very long time (6 weeks). Although the liquid culture techniques needs less time (21 days), it is still a long time [20]. A need for rapid and reliable method for diagnosis of TB is mandatory for the effective control of the TB disease.

Our results revealed that GeneXpert MTB/RIF has high sensitivity of 88.7% and specificity of 97.8% for detecting TB in pulmonary samples. For detecting smear negative culture positive cases, our results illustrated a sensitivity of 79.3% and specificity 99.5%, respectively. In our study twenty two out of 23 smear negative GeneXpert positive patients were having findings in their chest X- ray, so the sensitivity will increase to 95.9% if we include the X- ray finding in our consideration. The results obtained from our study go nearly in line with a meta-analysis from high TB burden countries which reported the pooled sensitivity of GeneXpert MTB/RIF in smear positive-culture positive pulmonary TB as 95.7%, and a sensitivity was 77.7% and specificity 99.6% for smear negative TB [21]. In low TB burden countries the pulmonary specimens demonstrated a pooled sensitivity of 89% and 67% (among total TB and smear-positive TB cases, respectively) and specificity of 99% for the detection of MTB [17]. These important findings, especially in a high TB burden country like Egypt, will help in rapid diagnosis of smear-negative TB cases especially those associated with significant X-ray findings and this will lead to a large improvement in TB control programs.

In our study the GeneXpert MTB/RIF method have proved 8 false-negative and 5 false-positive results, using the conventional culture methods as a gold standard method. From the 8 false negative specimens, 6 were smear-negative and 2 were smear-positive (+1). This was attributed to the low bacillary load in the examined specimens, as has been described previously that the GeneXpert MTB/RIF need higher bacillary load than the culture for detection of the organism. The Lower Limit of Detection (LLD) in the GeneXpert MTB/RIF from sputum samples (131 CFU/mL) is higher than culture (10 – 100 CFU/mL) [22]. The 5 false positive

Table 4. Evaluation of the GeneXpert MTB/RIF and the AFB smear microscopy as a diagnostic tool for pulmonary TB, using mycobacterial culture as the gold standard method

		Culture result			Performance			
		Pos	Neg	Total	Sensitivity	Specificity	PPV	NPV
		71	229	300	(95% confidence interval)			
Xpert MTB/RIF results								
total (300)	**+**	63	5	68	88.7	97.8	92.6	96.5
	-	8	224	232	(79.0 % to 95.0%)	(94.9% to 99.2%)	(84.0% to 96.7%)	(93.58% to 98.18%)
	total	71	229	300				
Smear positive (60)	**+**	40	4	44	95.2	77.8	90.9	87.5
	-	2	14	16	(83.84% to 99.42%)	(52.36% to 93.59%)	(80.78% to 95.97%)	(63.90% to 96.51%)
	total	42	18	60				
Smear negative (240)	**+**	23	1	24	79.3	99.5	95.8	97.2
	-	6	210	216	(60.28% to 92.01%)	(97.39% to 99.99%)	(76.34% to 99.39%)	(94.49% to 98.62%)
	total	29	211	240				
AFB Smear results								
	+	42	18	60	59.2	92.1	70.0	87.9
	-	29	211	240	(46.84% to 70.68%)	(87.86% to 95.28%)	(58.99% to 79.10%)	(84.58% to 90.61%)
	total	71	229	300				
P-value (**GeneXpert MTB/RIF overall versus AFB Smear**)					0.0001	0.01	0.0009	0.0005

Pos: Positive, Neg: Negative, PPV: Positive Predictive Value, NPV: Negative Predictive Value and CI: Confidence Interval

Table 5. Evaluation of the GeneXpert MTB/RIF and the AFB smear microscopy as a diagnostic tool for extra pulmonary TB, using culture method as the gold standard

		Culture result			Performance			
		Pos	**Neg**	**Total**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
		15	**105**	**120**		**(95% confidence interval)**		
GeneXpert MTB/RIF results								
Total (120)	**+**	12	0	12	80	100	100	97.2
	-	3	105	108	51.91% to 95.67%	96.55% to 100.00%		92.71% to 98.97%
	total	15	105	120				
Smear positive (6)	**+**	4	0	4	100	100	100	100
	-	0	2	2	39.76% to 100.00%	15.81% to 100.00%		
	total	4	2	6				
Smear negative (114)	**+**	8	0	8	72.7	100	100	97.1
	-	3	103	106	39.03% to 93.98%	96.48% to 100.00%		92.90% to 98.90%
	total	11	103	114				
AFB Smear results								
	+	4	0	4	26.7	100	100	90.5
	-	11	105	116	7.79% to 55.10%	96.55% to 100.00%		87.55% to 92.83%
	total	15	105	120				
P-value (**GeneXpert MTB/RIF total versus AFB Smear**)					0.004	0	0	0.039

Table 6. Frequency of anti-tuberculous drug resistance rate among the studied specimens

Specimen type	Total number of positive	Anti-tuberculous drug resistance		
		Rifampicin (RIF) resistance	Other drug resistant	Total No. (%)
Pulmonary	71	2	8	10 (14.1)
Extra Pulmonary	15	0	2	2 (13.3)
Total	86	2	10	12 (14.0)

Table 7. Evaluation of the GeneXpert MTB/RIF for the detection of RIF resistance, relative to phenotypic DST

	DST result			Performance			
	RIF-R	RIF-S	Total	sensitivity	specificity	PPV	NPV
	2	84		(95% confidence interval)			
GeneXpert results							
RIF-R	2	1	3	100	98.8	66.7	100
RIF-S	0	83	83	(15.81% to	(93.54% to	(22.18% to	
total	2	84	86	100.00%)	99.97%)	93.35%)	
After discrepancy resolution by sequencing results							
RIF-R	3	0	3	100	100	100	100
RIF-S	0	83	83	(29.24% to	(95.65% to		
total	3	83	86	100.00%)	100.00%)		

RIF: Rifampicin, S: Sensitive and R: Resistant

Table 8. Drug resistant specimens by phenotypic DST (n =12)

Specimen type	Specimen n	Anti-TB agent			
		RIF	INH	PZA	EMB
Pulmonary	1	S	R	S	S
	2	S	R	S	S
	3	R	R	R	R
	4	S	S	R	S
	5	S	R	S	S
	6	S	R	S	S
	7	S	R	S	S
	8	S	R	S	S
	9	S	R	S	S
	10	R	R	R	R
Extra-pulmonary	11	S	R	S	S
	12	S	S	R	S
Percent of mono drug resistance related to whole examined specimens (%)		2/86 (2.3%)	8/86 (9.3%)	2/86 (2.3%)	0/86 (0%)
Percent of MDR		2/86=2.3%			

RIF, rifampin; INH, isoniazid; PZA, pyrazinamide; EMB, ethambutol; R, resistant; S, susceptible

specimens in our study were collected from patients receiving anti-TB treatment at the time of testing. The same observation was reported by others [7,20]. The GeneXpert MTB /RIF method has a limited utility in patients under anti tuberculosis therapy as it will detect the genetic material of the remnant non-viable organism giving false positive results.

In our study, the sensitivity and specificity for the GeneXpert MTB /RIF method was evaluated for diagnosis of extra-pulmonary cases and results demonstrated sensitivity and specificity of 80% and 100%, respectively in overall specimens and 72.7% and 100%, respectively in smear negative cases. These results were in agreements with the results of a study done on 980 patients [23] who reported a total sensitivity of 77.6% and specificity of 99.9% for the GeneXpert MTB/RIF in the diagnosis of extra-pulmonary TB, while a sensitivity of 70.3% and specificity of 99.9% in smear negative cases. Nearly same results were

obtained recently [24] who reported a total sensitivity of 76.8% and 73.2% in smear negative and specificity of 99.1%. Meanwhile, the sensitivity of the AFB staining microscopy method was very low in our study 26.7% with a high significant statistical difference between it and GeneXpert MTB/RIF method (p= 0.004) this augment the role of the GeneXpert MTB/RIF in a rapid diagnosis of extra-pulmonary TB cases. The performance of GeneXpert MTB/RIF was found to be influenced by smear status not by TB prevalence [25] and these data support the finding of a large Meta -analysis [26].

Drug resistance in TB therapy is a major public health problem. Drug resistance arises due to improper use of anti-tuberculous drugs. The rate of drug resistance is highly varied in different areas around the world and even in the different areas of the same country as it is affected by multiple variable causes [27]. In our study MDR was detected in 2 out of 86 isolated strains (2.3%), higher rates of resistance were reported in other studies [28,29]. However, less rates of resistance (1.5%) were observed in San Diego country [20], and also 0% Rifampicin resistance among MTB positive cases in 2010-2012 was reported mainly from South India [30]. Nearly same results to our study were observed [5]. The possible reasons of a relative higher prevalence of drug resistance in our study may be due to mixing of new as well as recurrent cases, small sample size as well as the improper use of antibiotic in general in our country which lead to development of drug resistance.

The results of our study revealed that GeneXpert MTB/RIF demonstrated sensitivity and specificity for the detection of RIF resistance 100% (2/2) and 98.8% (83/84), respectively, using DST as the gold standard. Our results are in consistence with many recent studies [20,24,31].

Our results revealed that the RIF resistance was detected by GeneXpert MTB/RIF assay in two specimens which was also identified as RIF resistant by DST, in addition to one specimen that DST identified it as RIF susceptible. For the one specimen with discrepant GeneXpert MTB/RIF and DST results, DNA sequencing revealed that strain has an *rpoB* gene mutation codon 511 (CTG/CCG) (Leu/Pro) which was reported to be associated with low-level RIF resistance mutation. Incorporating the results of sequencing, GeneXpert MTB/RIF specificity and PPV increased to 100 (83/83) and 100 (3/3), respectively. In concordance with our results, sequencing revealed RRDR mutations in all discordant phenotypically susceptible strains [32] and also other studies reported the detection of low level mutations by sequencing which was not detected by DST [33,34].

Detection of Rifampicin resistance is regarded as a valid method for detection of MDR-TB as a large proportion of Rifampicin-resistant strains also has noticeable resistance to isoniazid (INH) the addition of this result to the previous results reinforce the use of GeneXpert MTB/RIF as prompt rapid diagnostic tool for early detection of Rifampicin resistance [5].

5. CONCLUSION

The obtained results of our study fulfilled the aim of the study and confirmed the diagnostic utility of the GeneXpert MTB/RIF in early diagnosis of pulmonary TB especially in patients with smear negative results with radiological and clinical findings highly suggestive of TB and for diagnosis of extra-pulmonary tuberculosis. Our findings also inforce the value of GeneXpert MTB/RIF assay in TB control program as a prompt rapid diagnostic tool for early detection of Rifampicin resistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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