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Original article

Evaluation of GeneXpert assay performance for pulmonary tuberculosis at King Abdulaziz University Hospital: A two years' surveillance study

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Abbreviations:
AFB: Acid fast stain
DST: Drug-susceptibility testing
KAUH: King Abdulaziz University Hospital
MDR: Multidrug resistance
MTBC:*Mycobacterium tuberculosis complex*
NTM: Non tuberculous mycobacterium,
PCR:Polymerase chain reaction
RIF: Rifampicin
TB: Tuberculosis

ABSTRACT

Background: Tuberculosis (TB) is one of the deadliest health problems. Accurate rapid diagnosis and prompt treatment are crucial for cure. The aim of this study was to investigate the role of GeneXpert PCR in the rapid detection of *Mycobacterium tuberculosis complex* (MTBC) directly from clinical samples and to determine the anti-TB drug resistance patterns in pulmonary tuberculosis. **Methods:** This is a cross-section analysis, involving a total of 92 patients with pulmonary TB (primary TB resistance); 37 Saudis and 55 non-Saudis. Respiratory specimens were processed, and examined by flurochrome stain. Culture and susceptibility testing was performed by VersaTREK , a liquid culture system. Adding, MTBCwas directly detected from the sample by GeneXpert PCR. **Results:** GeneXpert assay achieved 96 % sensitivity and 100 % specificity for the detection of MTBC in sputum. Resistance to pyrazinamide was (6.5%), followed by rifampicin (2.2%), streptomycin and isoniazid (1.1%). Mono-resistance to pyrazinamide and streptomycin (5.4%) and (1.1%) respectively. Multi-drug resistane (MDR)-TB was (1.1%) and there was no substantial difference in anti-TB resistance between Saudi and non-Saudi TB patients. **Conclusions:** The GeneXpert PCR is a very helpful tool for the detection of MTBC in respiratory specimens with a high sensitivity, specificity and accuracy within a shorter time as compared to conventional methods. **Recommendations:** Costs-benefit analysis of GeneXpert versus other recently deployed TB diagnostic systems may be required on a wider scale.

Introduction

Tuberculosis (TB), with about 10 million new infections and 1,8 million annual deaths, is one of the world's leading cause of morbidity and death [1,2]. Precise rapid diagnosis and prompt treatment are required for cure [3]. Global TB surveillance is facing two major challenges: the increasing rates of the human immunodeficiency virus (HIV) and the prevalence of drug resistance to TB [4]. Drug resistance to anti-TB usually increases and spreads due to mismanagement of TB cases. The primary resistance to TB results from transmission of resistant TB bacilli from an infectious case to another susceptible individual [5].

Millions of Muslims fly to the Kingdom of Saudi Arabia (KSA) every year in Hajj and Umrah. Many pilgrims come from high incidence TB countries such as South East Asia, Indian subcontinent and Africa. They live for at least a week, or a lot

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longer. Subsequently, TB transmission within KSA is enhanced due to overcrowding, physical exhaustion, heat stress and in many cases the suboptimal living conditions [6]. Local analysis of the prevalence of drug resistance strongly supports the use of the four-drug therapy protocol consisting of isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA) [7].

Continued monitoring of *M. tuberculosis* drug resistance patterns is of great importance in reducing the risk factors for MDR-TB infection in KSA [7]. The solid medium based drug-susceptibility test (DST) approach is slow and delays the identification of drug resistance *M. tuberculosis* with increased risk of incorrect diagnosis and potential spread of drug-resistant strains. These factors magnify the need for new detection and DST methodologies, including the use of liquid cultures [8]. Moreover, several direct techniques for MTBC DNA amplification from a wide range of samples have been licensed by the Food & Drug Administration (FDA). However, most of these fast tests do not provide full details on anti-TB susceptibility [9].

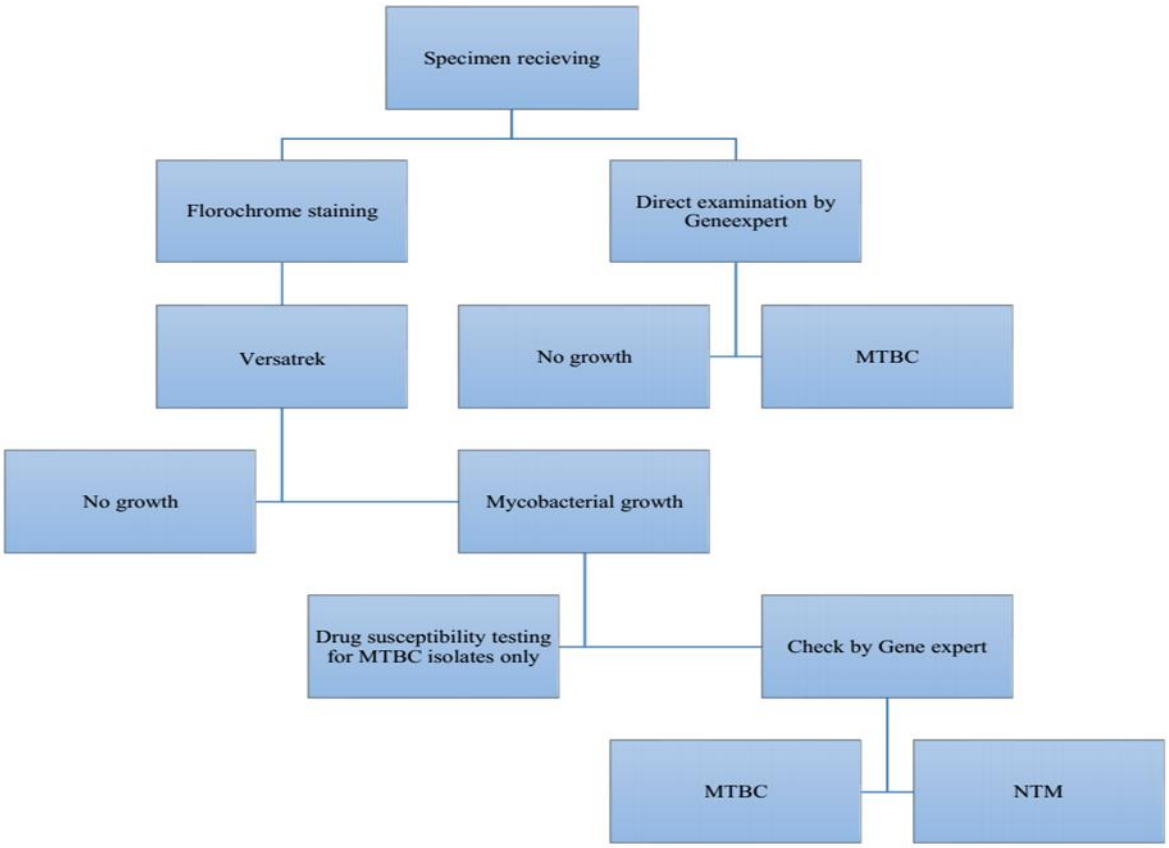
The objective of this study was to research the role of the GeneXpert PCR in the rapid detection of MTBC from clinical samples and to identify drug resistance patterns against anti-TB drugs in pulmonary TB at King Abdulaziz University Hospital, Jeddah.

Methods

The study was conducted at the King Abdulaziz University Hospital (KAUH) Clinical & Molecular Microbiology Laboratory in Jeddah, KSA. The study was conducted in compliance with the Ethical approval from, Faculty of Medicine, King Abdulaziz University (Reference Number: 276-17).

From July 2015-June 2017, all the clinical isolates used in this study have been obtained at KAUH. The 92 mycobacterial isolates used in the study have been isolated from cases of pulmonary TB (Primary Anti-TB Resistance); primary resistance is defined as resistance in newly diagnosed patients without prior history of anti-TB therapy or treatment for less than one month. The flow of sample processing was illustrated in **figure (1)**.

Figure 1. Flow of specimen processing.



Isolates identification

Clinical specimens were processed by standard laboratory protocols using florescent microscopy [fluorochrome stain (Fluo-RAL-Auramine staining kit) for Mycobacteria Detection (RAL-automated staining system-R.A.L. instruments, Montesquieu-33650 MARTILLAC-France)] and culture in the VersaTREK 528 (Trek Diagnostic Systems, Inc. Westlake, Ohio, USA) liquid culture system as a gold standard.

Direct MTBC detection from clinical specimens was done by GeneXpert PCR (Cepheid 904 Caribbean Drive Sunnyvale, CA 94089-1189-USA).

Drug susceptibility testing

Using results of GeneXpert, if the isolate is a MTB complex, drug susceptibility testing was conducted by VersaTREK (Remel™ Oxoid™ VersaTREK and Sensititre™ products) for rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide. Isolate in a drug-containing bottle is considered to be resistant if the detection time is equal to or within three days of the detection time of a drug-free control bottle [10].

Statistical analysis

Data were analyzed using Software for the Social Sciences Statistical Package (SPSS), version 18. To test the relation and/or discrepancy between categorical variables, Chi square test was used. Where appropriate, exact Fisher test was used. The mean, standard deviation and range were viewed as continuous variables. Student t test was used to compare between two groups. *P value* less than *0.05* was considered statistically significant. Sensitivity, specificity, accuracy, positive and negative predictive values were calculated.

Results

A total of 92 patients with confirmed pulmonary TB (primary anti-TB resistance) were examined and divided into 2 categories. The first group comprises 37 Saudis, with ages ranging from (15 to 87) years of age with mean ± SD (46.38± 22.29) years, and 24 (64.9 %) of whom were male, while 13 (35.1%) were female. The second group of patients with TB were 55 non-Saudis, ranging in age from 1 to 81 years with mean ± SD (41.42± 19.74) years, while 32 (58.2 %) were male and 23 (41.8 %) were female. Clinical samples were obtained from different clinical units: Emergency Unit (77.2%), Isolation Unit (13%), Private Unit (2%), Male Surgery Unit (2%) and Outpatient Department, Cardiac Care Unit, Medical unit and Day Care Unit were (1%) each.

GeneXpert PCR results for the detection of MTBC in different clinical specimens is presented in **table (1)**. The highest levels were for sputum specimen.

Mycobacterium tuberculosis complex detection by Gene-Xpert PCR is displayed in **table (2)**. The sensitivity, specificity, accuracy, PPV and NPV of Gene-Xpert PCR for the detection of MTBC in total respiratory samples were (88%), (100%), (90%), (100%) and (69%) respectively. In acid fast bacilli (AFB)-negative samples they were (47%), (100%), (74%), (100%) and (65%) respectively. The sensitivity, specificity, accuracy, PPV and NPV of Gene-Xpert PCR for the detection of MTBC in AFB-positive samples were (100%), (100%), (100%), (100%) and (100%) respectively.

Table 3 shows that the resistance to pyrazinamide was the most common form of resistance (6.5%), followed by the resistance to rifampicin (2.2%) and the INH and Streptomycin were 1.1% equally. Mono-resistance to pyrazinamide and Streptomycin were 5.4% and 1.1% respectively. Meanwhile, MDR-TB was 1.1%, and the percentage of any drug resistance was 8.7%. There was no substantial difference in resistance to anti-tuberculosis drugs between Saudi and non-Saudi TB patients.

Table 1. AFB staining results and MTBC detection by GeneXpert in different respiratory specimens

Types (No.) of specimen examined	No. (%) AFB staining results		Detection of MTBC by PCR	
			No. (%) Detected	No. (%) Not detected
Sputum (73)	Total sputum	73 (100%)	51 (69.9%)	22 (31.1%)
	AFB -ve	24 (32.9%)	5 (20.8%)	19 (79.2%) 17 NMT-2 MTBC
	AFB +ve	49 (67.1%)	46 (93.9%)	3 (6.1%) NTM
	+1	16 (21.9%)	13 (81.3%)	3 (18.8) NTM
	+2	15 (20.5%)	15 (100%)	0 (0%)
	+3	12 (16.4%)	12 (100%)	0 (0%)
	+4	6 (8.2%)	6 (100%)	0 (0%)
Tracheal aspirate (11)	Total TA	11 (100)	8 (72.7)	3 (27.3)
	AFB -ve	5 (45.5%)	2 (40%)	3 (60%)
	AFB +ve	6 (54.5%)	6 (100%)	0 (0%)
	+1	3 (27.3%)	3 (100%)	0 (0%)
	+2	1 (9.1%)	1 (100%)	0 (0%)
	+3	1 (9.1%)	1 (100%)	0 (0%)
	+4	1 (9.1%)	1 (100%)	0 (0%)
Bronchial wash (8)	Total BW	8 (100)	4 (50%)	4 (50%)
	AFB –ve	5 (62.5%)	1 (20%)	4 (80%)
	AFB +ve	3 (37.5%)	3 (100%)	0 (0%)
	+1	3 (37.5%)	3 (100%)	0 (0%)
Total specimens (92)			63 (68.5%)	29 (31.5%) 20 NTM & 9 MTBC

Table 2. MTBC detection of AFB-negative, AFB-positive by GeneXpert PCR.

AFB staining results	Detection of MTBC by PCR	
	No. (%) Detected	No. (%) Not detected
AFB-negative (34)	8 (23.5%)	26 (76.5%) 17 NTM & 9 MTBC
AFB-positive (58)	55 (94.8%)	3 (5.2%) NTM
AFB +1 (22)	19 (86.4%)	3 (13.6) NTM
AFB +2 (16)	16 (100%)	0 (0%)
AFB +3 (13)	13 (100%)	0 (0%)
AFB +4 (7)	7 (100%)	0(0%)
Total specimens (92)	63 (68.5%)	29 (31.5%) 20 NTM & 9 MTBC

Table 3. Anti-TB drug resistance patterns of MTBC in Saudi and non-Saudi TB- patients.

Pattern of anti-TB drug resistanc	Total resistance No. (%)	Resistance in Saudi No. (%)	Resistance in Non-Saudi No. (%)	*P-value*
Any resistance to RIF	2 (2.2%)	0 (0%)	2 (100%)	*0.41*
Any resistance to INH	1 (1.1%)	0 (0%)	1 (100%)	*0.60*
Any resistance to PZA	6 (6.5%)	1 (16.7%)	5 (83.3%)	*0.37*
Any resistance to streptomycin	1 (1.1%)	1 (100%)	0 (0%)	*0.42*
MDR (RIF+INH)	1 (1.1%)	0 (0%)	1 (100%)	*0.41*
Mono resistance to PZA*	5 (5.4%)	1 (20%)	4 (80%)	*0.34*
Mono resistance to streptomycin	1 (1.1%)	1 (100%)	0 (0%)	*0.22*
Any drug resistance	8 (8.7%)	2 (25%)	6 (75%)	*0.36*
One drug resistance	6 (6.5)	2 (33.3%)	4 (66.7%)	*0.66*
Two drug resistance	2 (2.2%)	0 (0)	2 (100%)	*0.24*
No drug resistance	84 (91.3%)	35 (41.7)	49 (58.3)	*0.36*

*Further analysis of those specimens in a later time revealed those specimens were *Mycobacterium bovis.*

Discussion

The global prevention of TB is still a challenging problem in terms of diagnosis, detection of drug resistance and treatment opportunities. Using rapid molecular tests to diagnose TB bacilli and anti-TB drug resistance plays a very important role in timely and proper treatment [11]. The WHO therefore proposed the application of these rapid molecular methods to screen MDR-TB patients quickly. In addition to rifampicin resistance (RIF), molecular approaches are used for MTBC diagnostics within 2 hours to ensure high sensitivity and precise characteristic. This can also be achieved directly from the sample, decrease the risk of occupational infection and prevent cross-contamination between clinical specimens using a closed disposable cartridge [12]. The current study therefore aimed at identifying patterns of anti-TB drug resistance and investigating the role of the GeneXpert method in the rapid detection of MTBC directly from clinical samples in cases of pulmonary TB.

Age and sex differences among Saudi- and nonSaudi-TB patients were not statistically significant in this study. Similar findings were found in previous studies [13,14], in 622 Primary TB patients surveyed, in Madinah Al-Munawara in Saudi Arabia, a seasonal overcrowded area during Hajj and Umrah, either nationality or gender was not associated with MDRTB [13]. However, the frequency of non-Saudi was higher than that of Saudi [15], according to a previous report. The incoherence in TB incidence in different KSA regions may have been attributable to differences between studies. For example, Jeddah (sea and airports for pilgrims arriving in Mecca) has higher levels which may have been caused by the pilgrim influx [16]. Another study found that males were more commonly diagnosed with pulmonary tuberculosis than females [17]. It may have to do with KSA customs which include women wearing hijab covering their faces. Many women are housewives with lower incidence of exposure to infectious droplets than males. Furthermore, males spend much of their time outside their homes owing to their work arrangements or with mates. Smoking is an important factor for men as they have a higher TB rate [18].

In this recent study, the 96% sensitivity and 100% specificity for MTBC sputum detection were achieved. The most surprising finding is that 3 of the 16 specimens were not described by GeneXpert. These were positive "+1 AFB" smear specimens, and subsequently labelled as NTM. In the same manner, a previous study in Riyadh found that AFB is not identified by Gene Xpert 's test since it was NTM [12].

Earlier reported GeneXpert sensitivity variations ranged from 57% to 96% for negative AFB samples [19-22]. The difference in patient selection criteria between trials that would involve only investigation of strong suspects of TB patients can explain different sensitivities [23]. 17/26 samples have been identified in the current analysis to be NTM. Fortunately, GeneXpert system can differentiate between MTB and NTM in smear-positive cases of acid-fast bacilli (AFB) [24]. The most common form

of resistance in the current study was pyrazinamide resistance (6.5%) followed by rifampicin resistance (2.2%) and then isoniazid and streptomycin resistance (1.1%) respectively. Mono-resistance to two anti-TB drugs only was reported, which were pyrazinamide (5.4%) and streptomycin (1.1%). These results were very striking for the laboratory consultants at the time, and the specimens were retested for drug susceptibility when repeated PZA resistance was confirmed. Then, after a certain time, the specimens were re-analyzed with a different technique, they were tested with Hain PCR (MTBDR-CM kit), which all proved to be a PZA resistant Mycobacterium bovis.

In contrast, another study was carried out at the King Faisal Specialist Hospital and Research Center in Riyadh, Saudi Arabia; mono-resistance to streptomycin (8.1%), isoniazid (5.4%) and rifampin (1%) was found. MDR-TB was contained in (1.8 %) [24]. Adding to this was the highest prevalence of anti-tuberculosis resistance for isoniazid followed by streptomycin and MDR-TB rifampicin (6.7%) in a separate study [25]. A further research found isoniazid mono-resistance (1.8%), rifampicin (1.4%), streptomycin (1.9%), and pyrazinamide (2.1%) [13]. In 2014, **Somily and his co-investigators** announced that the isoniazid resistance rate was (10.6%), rifampicin was (2.0 %), streptomycin was (6 %) and MDR (0.7 %) was [14] according to a preliminary Saudi analysis on TB drug resistance. **Altawfiq et al.** found that the prevalence of INH anti-tuberculosis resistance accompanied by streptomycin, rifampicin and MDR-TB remained high Dahran, Saudi Arabia (6.7%) [26]. The percentage variability of anti-TB drug resistance in Saudi Arabia depends on the time, patient numbers, the nationality of participant and the place of the study [27]. A real need exists for continuous updated monitoring on the national level as well as in each Saudi region.

In the current report, the percentage of MDR-TB was 1.1 % with no substantial difference in any form of resistance to anti-tuberculosis drugs between Saudi and non-Saudi TB patients. These findings were consistent with **Elhassan et al.** which found that MDR-TB was not associated with either nationality or gender [13]. This would lead to designating Saudi Arabia's health strategy.

In conclusion, GeneXpert is still a sensitive method for the rapid diagnosis of pulmonary tuberculosis, particularly in smear positive cases and in sputum specimens. GeneXpert may be a rapid, sensitive and cost-effective diagnostic tool for pulmonary TB in countries endemic to TB, which may have a positive clinical impact. Low levels of resistance to all anti-TB drugs with no major difference between Saudi and non-Saudi patients were observed.

Recommendations

In order to enforce the current TB prevention and management plans in KSA, a continuous monitoring system is required to track resistance patterns in MTBC isolates. Costs-benefit analysis of GeneXpert versus other recently deployed TB diagnostic systems may be required on a wider scale.

Limitations of the study

The size of the sample is very small. Samples with NTM lowered the overall sensitivity of GeneXpert. Just one sample per patient was used, we may increase the overall yield of bacilli and the sensitivity of GeneXpert by mixing more than one sample for the same patient. The majority of isolates were also susceptible, so that the patterns of resistance were not extensively studied.

Conflict of interest

Authors declare no conflict of interest and no received fund from any drug company to support this research

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