Molecular Detection of RIF Resistance in Pulmonary and Extrapulmonary Specimens in Misan Province–South of Iraq

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ABSTRACT

Background: Tuberculosis remains an important public health problem especially in the developing world. The tuberculosis burden is worsened by the emergence and spread of multi-drug resistant bacteria. The aim of current study was to evaluate detection of Mycobacterium tuberculosis in respiratory and non-respiratory specimens and mutations associated with rifampicin (RIF) resistance directly from specimens in less than 2 hours by using GeneXpert MTB/RIF.

Method: A total of 511 patients attended to the TB center in Misan/Iraq from January 2016 to February 2018. These patients were clinically diagnosed or suspected to have TB. The samples were examined by GeneXpert assay. For extra pulmonary fluid samples, the same test was done.

Results: Out of 511 cases, 90(17.6%) were detected as having M. tuberculosis─ 86(95.5%) were rifampicin sensitive and 4(4.4%) were rifampicin resistant. Also, 79(87.7) were pulmonary samples and the remaining 11(12.2) cases were extrapulmonary specimens (lymph node aspirate, pleural fluid, saliva, abscess and bronchoalveolar lavage).

Conclusions: GeneXpert assay is a new rapid molecular test in the detection of *Mycobacterium tuberculosis* directed from pulmonary and extra pulmonary specimens and detection of resistance to antibiotics(Rifampicin).

Keywords: *GeneXpert assay, MTB, Rifampicin, Drug resistant, Extra pulmonary.*

Introduction

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* (MTB). It spreads from person to person predominantly through an airborne route. It remains a major global health problem as it causes ill-health among millions of people. After the human immunodeficiency virus (HIV), TB ranks as the second leading cause of death from an infectious disease worldwide [1,2]

Worldwide, there are estimated 9 million new cases and 1.4 million deaths caused by *Mycobacterium tuberculosis* (MTB) infections [3]. These numbers are increasing due to the increasing emergence and spread of multi-drug resistant *M. tuberculosis* strains (MDR-TB). The latter are now a serious threat for public health control systems and do not have effective treatment regimens [[4]. Rifampicin is a very important component of the current tuberculosis treatment regimen and has proved to be effective to both susceptible strains and strains resistant to streptomycin and isoniazid [5]. Drug resistance in MTB has been characterized by a number of mutations in genes that are involved in drug targets/ metabolism. Mutations in rpo B and kat G genes of MTB have been shown to be responsible for resistance to rifampin (RIF) and isoniazid (INH), respectively [6]. Ninety-five percent of mutations for RIF resistance are located in 81-bp region of the *rpo*B gene that codes for the β subunit of RNA polymerase. Mutations in codon 315 of the *kat*G [7].

Rapid detection of drug resistance is crucial in choosing the most effective treatment to avert morbidity and mortality of infected individuals and reduce

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the risk of MDR tuberculosis transmission [5]. Xpert MTB/RIF assay is a new rapid molecular test that was asserted that it was able to overcome many of the current operational difficulties in TB diagnosis. WHO endorsed Xpert MTB/RIF assay in 2010 for use in TB prevalent resource limited countries as a first line diagnostic test for rapid diagnosis of TB in HIV infected patients or for management of MDR-TB suspect [8].

In this study, we aimed for detection of Mycobacterium tuberculosis in respiratory and non-respiratory specimens and mutations associated with rifampicin (RIF) resistance directly from specimens in less than 2 hours by using GeneXpert MTB/RIF (Cepheid GeneXpert® System, USA).

Material and Method

Clinical samples: In total, 511 specimens were included in the study. They originated from patients with suspected TB between January 2016 to February 2018 at TB center in Misan Governorate, Iraq. A total of 90 samples (79) respiratory and (11) non-respiratory specimens) that were determined as positive for *M. tuberculosis* complex by Xpert MTB/RIF (Cepheid GeneXpert® System, USA) were included in the study.

Procedures for Xpert assay: Xpert MTB/RIF testing was performed on samples according to the manufacturer's recommendations. The Xpert assay sample reagent (containing NaOH and isopropanol) was added in a 1:3 ratio to the tubes to kill the mycobacteria and liquefy the sample. The mixture was vigorously shaken and allowed to sit for 15min before being shaken again and allowed to sit for another 5min. Finally, 2mL were pipetted into the Xpert assay cartridge and inserted into the GeneXpert instrument for polymerase chain reaction (PCR) testing. The measurement and analysis were conducted automatically and reported by the GeneXpert Dx software.

Fluid samples from non-respiratory sites were centrifuged at 400xg for 15 minutes then the deposit was dealt with exactly like sputum samples. The tubes were incubated at room temperature for at least 15 minutes during that they were shaken manually twice then 2ml of this suspension was aspirated using Pasteur pipette and loaded into the GeneXpert cartridge for PCR test [9]).

Results

A total of 511 specimens were run in the Xpert MTB/RIF assay. MTB was detected by GeneXpert in 90 (17.6%) specimens, while MTB was not detected in 421 (82.38%) specimens (Table 1). Rifampicin resistance among MTB detected cases was observed by GeneXpert in 4 (4.4%) out of 90 MTB cases (Table 2). Among 90 (17.6) MTB detected cases 50 (55.6) were males and 40 (44.4) were females (Table 3).

The pulmonary specimens were detected in GeneXpert assay 75 (84.3), and extra-pulmonary specimens (plural fluid, lymph node aspirate, saliva, abscess, bronchoalveolar lavage) were detected in GeneXpert assay 11 (12.2) (3,1,1,2,4), respectively, (Table 4).

Table 1: Detection of MTB in pulmonary and extra-pulmonary specimens by using GeneXpert MTB/RIF assay

No. of Specimens	MTB detected (%)	MTB not detected (%)
511	90 (17.6)	421(82.38)

Table 2: Rifampicin resistance among MTB detected cases

MTB detected (n=90)		
No. of samples	**RIF resistance detected (%)**	**RIF not detected (%)**
90	4 (4.4)	86(95.5)

Table 3: Sex and age distribution among positive cases

Age Group/yr	Males n (%)	Females n (%)	Total n (%)
<25	6	11	17
25−34	10	8	18

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35−44	4	5	9
45-54	15	7	22
55-64	12	6	18
>65	3	3	6
Total	50 (55.6)	40 (44.4)	90(100)

Table 4: According types of specimens (pulmonary and extra-pulmonary specimens)

Types of samples Total	Sputum n (%)	Pleural fluid n (%)	Lymph node aspirate n (%)	Saliva n (%)	Abscess n (%)	Bronchoalveolar lavage n (%)
90	79 (87.7)	3 (3.3)	1 (1.1)	1(1.1)	2 (2.2)	4 (4.4)

Discussion

This study demonstrated that the Xpert MTB/RIF assay system can rapidly detect the presence of *M. tuberculosis* and identify the mutations most frequently associated with rifampin resistance directly from sputum samples.

In this study, GeneXpert assay detected M. tuberculosis in 90(17.6%) of cases. In another study, [10] detected *M. tuberculosis* in 283(94.33%) of samples out of 300, which disagreed with the findings of this study. GeneXpert MTB/RIF assay detected 4(4.5%) of MTB positive cases resistant to rifampicin therapy, which was very close (agreed) to what was found by [11], who reported a prevalence for Rifampicin resistance rate of 6.9%, and by [12] who reported 3.1% in Iran. However, [13] detected 20(14.2%) rifampicin-resistant cases out of the total positive MTB cases which disagreed with the findings of our study. This discrepancy in results could be attributed mostly to existence of mixed bacterial strains in sputum sample particularly in retreated patients, or due to gene mutations in MTB especially *rpo* B gene [14]. In our study, among 90 clinically suspected pulmonary and extra pulmonary tuberculosis cases, 50(55.6%) were males and 40(44.4%) were females. Another study, [15] reported that out of 107 pulmonary tuberculosis patients, 64(59.81%) were males and 43(40.19%) were females, this was almost similar to our study. The reason of higher male tuberculosis cases than female cases might be explained by the fact that males are actively populated in the community and may come in contact with TB infected persons more frequently, and the associated habits of alcoholism and smoking among the males which may imply an increased susceptibility of males to respiratory illnesses. But female members still reside at home and, therefore, the chance of exposure is comparatively less. Thus, it is recommended to use the GeneXpert assay to prevent prevalence MDR-TB and provide sufficient quantity of GeneXpert kits to examine all the negative and positive samples that attended the tuberculosis centers.

Conclusion

The current study proved the high incidence and prevalence rates of TB in Misan province. The GeneXpert can be rapid and helpful assay for the diagnosis of rifampicin resistant TB that needs minimal technique and can be operated by non-specialist laboratory staff. Furthermore, GeneXpert can provide results in a short period of time, as it is not necessary to wait for smear results like in the traditional method. As a result, treatment can be started more quickly.

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Ethical Clearance: The study was approved by the Research Ethics Committee at College of Medicine/ University of Misan, Iraq.

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Conflict of Interest: None to declare.

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