The Yield of Genexpert in Pulmonary Tuberculosis & Rifampicin Resistance.

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ABSTRACT:

BACKGROUND :

Earlier and improved tuberculosis (TB) case detection, including smear-negative disease & expanded capacity to diagnose multidrug-resistant tuberculosis (MDR-TB) are global priorities for TB control. The development of the Genexpert of mycobacterium tuberculosis and rifampicin resistance (MTB/RIF) assay for the Genexpert platform was completed in 2009 and is considered an important breakthrough in the fight against tuberculosis (TB). For the first time, a molecular test is simple and robust enough to be introduced outside conventional laboratory settings.

Genexpert MTB/RIF detects M. tuberculosis as well as rifampicin resistance-conferring mutations using three specific primers and five unique molecular probes to ensure a high degree of specificity. The assay provides results directly from sputum in less than 2 hours. It is commercial real-time polymerase chain reaction (PCR) assay (Xpert™ MTB/RIF).

OBJECTIVE:

To assess the accuracy of the Cepheid Xpert™ MTB/RIF in the detection of M. tuberculosis from respiratory samples in the setting of data collected from TB center in Medical city complex.

MATERIALS& METHODS:

This is primarily laboratory -based study, used stored data from a previously reported retrospective evaluation of 56 patients suspected of having TB from clinical point of view.

9 patients subjected to the examination of sputum & broncoalevoaler lavage (BAL) at the same time rendering the total specimen number about 65 specimens. The results of Genexpert & RIF sensitivity for each patient were discussed &compared with that of direct AFB smear & culture of sputum and broncoalevoaler lavage (BAL).

RESULTS:

The result s of Gene xpert was compared with direct smear microscopically examination which considered as a primary diagnostic test although the highly sensitivity of Genexpert in diagnosing viable tubercle bacilli ,additionally it diagnose the the non-viable one with the same efficiency, such a feature considered an Achilles tendon of this test.The study revealed high sensitivity& extraordinary specificity in diagnosing TB making this technology the most rapid,simple&accurate diagnostic test of TB .

CONCLUSION:

The results demonstrate that rifampin-resistant M. tuberculosis can be detected in DNA isolated from sputum samples in a single-tube assay that takes less than 3 h to perform; the assay is extremely specific and extraordinarily sensitive.

More over the assay is simple to perform and readily automatable for high-throughput screening.

KEYWORD: (xpert™ mtb/rif)- genexpert in pulmonary tuberculosis&rifampicin resistance.

INTODUCTION:

Tuberculosis (TB) is a leading public health problem worldwide causing 9 million active disease cases and 2 million deaths annually. Delayed diagnosis and incomplete or improper treatment of TB patients leads to the evolution of drug-resistant strains of *Mycobacterium tuberculosis*, including multidrug-resistant (MDR) and extensively drug-resistant TB (XDRTB). [1]

Active Tuberculosis Sputum microscopy and culture in liquid medium with subsequent drug-susceptibility testing are currently recommended as standard methods for diagnosing active tuberculosis. A new molecular diagnostic test

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called Genexpert MTB/RIF assay detects *M. tuberculosis* complex within2 hours, with an assay sensitivity that is much higher than that of smear microscopy. [2]

Drug-Resistant Tuberculosis The current standard for first-line drug-susceptibility testing is an automated liquid culture system, which requires 4 to 13 days for results. Commercial molecular line-probe assays can

Yield results in 24 hours, once they have been validated against automated liquid culture. [3]

The World Health Organization (WHO) recommends that standard drug-susceptibility testing be performed at the same time that the Genexpert MTB/RIF assay is performed to confirm rifampin resistance and the susceptibility of the *M. tuberculosis* isolate to other drugs [4]

The Genexpert diagnostic system was originally developed by Cepheid Inc. in 2009, for the detection of anthrax. [23] And was deployed for this purpose by the United States Postal Service in mail sorting facilities. This application required the development of a self-contained, fully integrated and automated platform that could be operated with minimal technical expertise. [5]

Genexpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, CA).This automated system employs real time PCR and molecular beacon probes to determine the presence of *M. tuberculosis* complex DNA as well as *rpoB*gene mutations conferring rifampin (RIF) resistance rapidly and with high accuracy of both smear positive and smear negative samples.[5,6,7] Rifampicin resistance is particularly amenable to rapid molecular detection since >95% of all rifampicin resistant strains contain mutations localized within the 81 bp core region of the bacterial RNA polymerase *rpoB*gene, which encodes the active site of the enzyme . [8] Moreover, mutations that occur in this region are highly predictive of rifampicin resistance, whereas susceptible isolates almost always have the same wild-type nucleotide sequence. [18, 19] In addition, the *rpoB* core region is flanked by *Mycobacterium tuberculosis*- specific DNA sequences. Thus, it is possible to test for *M. tuberculosis* and for rifampicin resistances simultaneously, by targeting a single amplicon generated using PCR technology. Moreover, Rifampicin resistance is strongly, although not invariably, indicative of MDR-TB (defined by concomitant resistance to isoniazid – another key anti- tuberculosis agent). Resistance to isoniazid, by contrast, is conferred by mutations in a number of genes and is a poor marker of MDR-TB since isoniazid mono resistance is common. The specificity of the MTB/RIF test in the diagnosis of TB has been shown to be very high (97–100%) in demonstration studies coordinated by the Foundation for Innovative New Diagnostics (FIND). [20, 21]

Taking culture as the gold standard, the sensitivity is > 95% for direct sputum smear-positive samples, and varies between 65% and 77% if direct sputum smear microscopy is negative,[20,22] with an incremental gain in sensitivity when the number of tests is increased from one to three. While a negative MTB/RIF test result does not exclude a diagnosis of TB, the test is much more sensitive than smear microscopy in detecting bacteriologically positive pulmonary TB. This is particularly important among human immunodeficiency virus (HIV) infected patients.[23]The MTB/RIF test should be used as the initial diagnostic test in individuals suspected of having (*MDR or*) HIV-associated TB' Any test result needs to be interpreted according to the prevalence of MDR-TB. Given the 95% sensitivity and 98% specificity of the MTB/RIF test in the detection of RMP resistance.

AIM OF THE STUDY:

To study and assess the accuracy of the Cepheid Xpert™ MTB/RIF resistance in the detection of M. tuberculosis from respiratory samples in the setting of data (Respiratory samples) collected from TB center in Medical city complex.

MATERIALS & METHODS:

This is primarily laboratory-based study, used stored data from a previously reported. Retrospective evaluation of 56 patients (65 samples) suspected of having TB from clinical point of view .29 patients found to be positive by direct sputum examination, **20** patients found to be positive by sputum culture. Both subgroups are subjected to examination by Gene Xpert MTB/RIF for detection of presence of *Mycobacterium tuberculosis*& rifampin (RIF) resistance in same kit.

In this retrospective study the patients was divided into 3 sub-groups:

1. The 1st group selected according to clinical features (fever.cough,weightloss,night sweat).

2 .The 2nd group subjected to sputum examination (sputum direct microscopical examination,sputum culture).

3. The 3rdgoup subjected to bronchoaleviolar lavage (direct sample microscopical examination, specimen culture)

The patients sample was gathered randomly from the file records of TB center in medical city complex to the period extended from January2013-june 2013(about 6 months).

The sputum specimen of all patients were collected & introduced into the Gene Xpert MTB/RIF instrument by laboratory staff of TB center, which utilizes molecular beacon technology to detect DNA sequences amplified in a hemi-nested rt-PCR assay.

Five different nucleic acid hybridization probes are used in the same multiplex reaction.

Each probe is complementary to a different target sequence within the *rpoB*gene of rifampicin-susceptible *M. tuberculosis* and is labeled with a different colored fluorophore.

The assay utilizes single-use plastic cartridges with multiple chambers that are preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing DNA extraction heminestedrt-PCR.

Clinical sputum samples (or decontaminated sputum pellets) are treated with a sodium hydroxide and isopropanol-Containing sample reagent (SR). The SR is added to the sample (currently recommended at a3:1 ratio for sputum pellets and a 2:1 ratio for unprocessed sputum samples) and incubated at room temperature for 15 min. This step is designed to reduce the viability of *M. tuberculosis* in sputum at least 106- fold to reduce biohazard risk[(42)] , is then manually transferred to the cartridge which is loaded into the Genexpert instrument. Subsequent processing is fully automated.

The standard user interface indicates the presence or absence of *M. tuberculosis* and the presence or absence of rifampicin resistance, by detection mutations in the *M. tuberculosis rpoB*gene in what is called molecular beacon technology.

Sensitivity and specificity was calculated according to the following formulae:

Sensitivity=No. of true positive/No. of true positive + No. of false negative. = No. of true positive / total No. of sick individual in population.

Specificity=No. of true negative/No. of true negative + No. of false positive. =No. of true negative/total No. of well individual in population.

RESULTS:

In this retrospective study, 56 patients (65 samples) were from patients who provided more than one sample (sputum, bronchial) samples all analyses were presented.

The results of Genexpert of each same group was compared with direct microscopically examination & culture of different samples.

In this retrospective study tuberculosis seems to affect male slightly predominantly, male to female ratio (1.3:1).

Of (56) samples processed (29) sample yielded M.Tuberculosis by sputum direct microscopical examination where's only (20) samples were positive by culture of sputum,

The Xpert™ MTB/RIF assay detected (41) patients positive M.Tuberculosis, (36)patients were RIF susceptibility positive , table (1).

All of smear- positive, culture-positive (19/19 100% & (5/6) 83% of smear- negative, culture –positive specimens were identified by the direct Gene Xpert test as having TB DNA.

The last case unidentified by Genexpert which is smear negative is considered to be as a false negative according to result of direct smear microscopical examination which considered as a primary diagnostic test although the highly sensitivity of Genexpert in diagnosing live tubercle bacilli, additionally it diagnose the dead one with the same efficiency.

This study revealed 5 cases; smear positive, Genexpert positive to be as rifampicin resistant while the other 36 cases are sensitive to rifampicin.

.There is 4 false-positive results, both smear- and culture-negative, that were collected from the patients who were strongly clinically suspected of M. tuberculosis.

Regarding BAL results Direct microscopical examination revealed (5) positive results while specimen culture revealed (4) positive cases.

All of smear positive, culture positive(3/3) 100%, 1 case smear negative, culture negative found

Genexpert positive(false positive)&1 case smear negative ,culture positive , found to be negative by Genexpert.

Sensitivity specificity of Genexpert in comparison of smear positive ,culture positive sputum specimen were 87%,95% respectively

Sensitivity and specificity of Genxpert in comparison of smear negative, culture positive 85%, 100% respectively.

In general view, Compared to the culture results of sputum, sensitivity of Genexpert assay was (96%) while specificity was (80%).
Sensitivity&specificity of Genxpert in comparison of smear positive ,culture positive BAL specimen were 100%,100% respectively

Sensitivity&specificity of Genxpert in comparison of smear negative, culture positive 75%, 100% respectively.
In general, compared to, the culture result of BAL the sensitivity result was (100%), while specificity was (75%).

Table 1: Showing numbers of patients subjected to sputum examination compared with subjected Genexpert.

	Types of Lab. Examination.			
	Sputum examination		Genexpert.	
	Direct microscopically examination	Sputum culture	Mycobacterium tuberculosis.	Rifampicin resistance
No. of patients.	29	20	41	36

Table 2:Comparisons of Genexpert positive results in relation to sputum microscopically examination (the latter used as standard value).

Types of sputum examination	Results	Genexpert positive results
Direct Smear Positive- Sputum culture positive	19	19
Direct Smear Negative- Sputum culture positive	6	5
Direct Smear Negative - Sputum culture Negative.	4	4

Table 3: Number of patients subjected to BAL examination compared with that subjected to Genexpert.

Types of examination	Bronchoalveolar lavage (BAL)		Genexpert.	
	Direct microscopically exam.	BAL culture	Mycobacterium tuberculosis	Rifampicin resistance
No. of patients	5	4	41	36

Table 4: Comparisons of Genexpert positive results in relation to BAL microscopically examination culture (the former used as standard value).

Type of BAL examination	Results	Genexpert positive
Direct Smear Positive- Sputum culture positive	3	3
Direct Smear Negative- Sputum culture positive	1	--
Direct Smear Negative - Sputum culture Negative.	1	1

DISCUSSION:

This is the first study to date in Iraq in which; smear microscopy used as the primary diagnostic test of tuberculosis in addition to MTB/RIF Xpert technology.
The results demonstrate that rifampin-resistant M. tuberculosis can be detected in DNA isolated from sputum samples in a single-tube assay that takes less than 3 hours to perform; the assay is extremely specific and extraordinarily sensitive.
Moreover the assay is simple to perform and readily automatable for high-throughput screening in high-burden countries. The distinction between smear –negative and smear-positive cases can be particularly important, Smear-positive cases poses an increased clinical, infection control and public health risk because of their higher disease burden and infectiousness. Smear-negative cases mark patients who might not have been identified and treated if sputum microscopy was the sole diagnostic modality.,
The results that are obtained from the assay indicate whether a patient is infected with M.Tuberclous & whether the tube of bacilli is present in the sample, and whether the bacilli are rifampin resistant.

Because all multidrug-resistant M. tuberculosis strains are rifampin resistant the results of the assay enable an immediate decision to be made as to whether to prescribe a more rigorous course of antibiotic treatment. Furthermore, the assay provides quantitative results, which could eliminate the need to perform sputum microscopy for routine tubercular Implementation of Gene xpert technology and rapid TB diagnostic and drug susceptibility testing may optimize national TB program operations throughout resource limited. Settings. [33]

In this retrospective study the result of Genexpert sensitivity and specificity in comparison sputum specimen in smear positive, culture positive was about 87%, 95% respectively, for sputum negative, culture positive specimen sensitivity and specificity were 85%, 100% respectively.

Sensitivity and specificity of Genxpert in comparison of smear positive, culture positive BAL specimen were 100%, 100% respectively

Sensitivity and specificity of Genxpert in comparison of smear negative, culture positive 75%, 100% respectively

Other studies performed by chang et al, who found the sensitivity& specificity 75%,93% respectively, another study by Lesley Scott ,who found , sensitivity & specificity for sputum positive, culture positive specimen about 95%,95% respectively, Sputum negative, culture positive specimen 80%, 90% respectively.

In another study done by Catharina C. Boehme & published in The New England Journal of Medicine among patients with culture-positive tuberculosis the overall sensitivity of the MTB/RIF test was 97.6%. The sensitivity was 99.8% for smear- and culture-positive cases and 90.2% for smear-negative, culture-positive cases, with no significant variation in overall sensitivity across sites.

Dr. Cengiz Cavusoglu found sensitivity& specificity of Smear-positive, culture- positive specimen 100 %, 100 respectively, for Smear-negative, culture- positive specimen74.2%, 99.4% respectively.

CONCLUSION:

.The results demonstrate:

1- That rifampin-resistant M. tuberculosis can be detected in DNA isolated from sputum samples in a single-tube assay that takes less than 3 h to perform.

2- The assay is extremely specific and extraordinarily sensitive. More over the assay is simple to perform and readily automatable for high-throughput screening.

3- Because all multidrug-resistant M. tuberculosis strains are rifampin resistant the results of the assay enable an immediate decision to be made as to whether to prescribe a more rigorous course of antibiotic treatment.

4- Furthermore, the assay provides quantitative results, which could eliminate the need to perform sputum microscopy for routine tuberculosis screening.

Recommendations:

1-Genexpert MTB/RIF should be used as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB.

2. Genexpert MTB/RIF may be considered as a follow-on test to microscopy in settings where MDR-TB or HIV is of lesser concern, especially in further testing of smear-negative specimens.

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