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ORIGINAL ARTICLE

Gene Xpert MTB/RIF versus BACTEC MGIT 960: Two Diagnostic Modalities for Recovery of Mycobacterium Tuberculosis from Pulmonary Cases

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ABSTRACT

***Background**: Mycobacteriology laboratory confirmation of tuberculosis stands as a key part in diagnosis, being a global and public health threat. National Tuberculosis Control Programs are endeavored by challenges of infection, dissemination and multidrug resistance behavior. Accurate and rapid diagnosis is mandatory to cut this vicious circle. The objective of this study is to assess the efficiency of Gene Xpert MTB/RIF in comparison to BACTEC MGIT 960 in diagnosis of mycobacterium tuberculosis and rifampicin resistance. **Methodology:** eighty patients were enrolled in this cross-sectional study from two Egyptian tertiary care hospitals. Early-morning sputum specimens were collected, followed by decontamination liquefaction technique, Ziehl- Neelsen staining and processing in Gene Xpert MTB/ RIF and MGIT 960. **Results**: Seventy-four specimens were positive for Mycobacterium tuberculosis by both MGIT 960 and Gene Xpert MTB/ RIF, only 66 were positive by ZN smear. **Conclusion**: This study unveiled the truthful accurateness of Gene Xpert MTB/RIF. The best sensitivity and specificity for diagnosis of MTB and rifampicin resistance in a single-sputum test, coupled with short turnaround time besides total automation potentiate the utility of Gene Xpert MTB/RIF as a diagnostic tool, which should be integrated in schematic approach to improve the management of pulmonary tuberculosis cases.*

INTRODUCTION

The slowly-declining tuberculosis (TB) incidence rate of 1% per year, remains an alarming sign, making it unbearable to reach the 2050. TB eradication target, defined as less than one TB / 1000000 population [1].

Each year approximately 580000 new multidrug resistance/rifampicin resistance-TB (MDR/RR-TB) cases appear among new and retreated TB cases, with overall 250000 deaths. Egypt is ranked by WHO as one of the middle/low level of TB incidence countries. National TB programs stated that about one half of TB patients had extended drug resistance/rifampicin resistance-TB (MDR/RR-TB) and approximately one fourth with XDR-TB, had a successful treatment outcome[2].

Conventional diagnostic methods for mycobacterium TB (MTB) are slow and/or lack sensitivity[3]. The outstanding recommendations of WHO introduced a stepwise methodology especially in middle and low income societies, using a liquid medium for culture and drug sensitivity test (DST)[4].

Rapid diagnostic methods allowing accurate susceptibility testing of first-line and second-line drugs are life-saving for early diagnosis of MDR-TB and XDR-TB for starting effective treatment [5, 6].

Bactec MGIT 960 is an automated nonradiometric non-invasive system that utilizes the oxygen sensor fluorescence for mycobacteria growth Detection in 13.3 days. It has a higher sensitivity comparing with other conventional solid media[7].

Gene Xpert MTB/RIF assay is an automated, closed-cartridge system, easy to operate and use friendly. It is based on a hemi nested real-time polymerase chain reaction (PCR) assay utilizing five molecular beacons technology spanning the *rpo*B gene 81-bp rifampicin resistance-determining region (RRDR). With concomitant diagnosis of MTB and susceptibility to rifampin, it stands as a surrogate indicator for multidrug MDR- TB[8]. The results are attained in 2 hours[9].

This study was scheduled to assess the efficiency of Gene Xpert MTB/RIF in comparison to MGIT 960 in diagnosing pulmonary tuberculosis cases and rifampicin resistance.

METHODOLOGY

Study design and setting:

This was a cross sectional study conducted over one-year duration. Specimen collection was done in Chest Department Zagazig University Hospitals and General Chest Hospital. The University Ethical Committee approved this study. Full written consent was taken from all enrolled patients before either history taking or specimen collection.

Patients:

This study included 80 clinically-suspected pulmonary TB cases (to be confirmed by mycobacteriology lab) from both hospitals' outpatients at Zagazig University Hospitals and Chest Hospital, Zagazig, Sharkia Governorate, Egypt, where clinical, radiological and microbiological assessment and treatment take place. The diagnosis of pulmonary TB was performed, as defined by National TB Control Program of Egypt, [10]

Clinical diagnosis of T.B.:

• Persistent cough of 2 weeks or more or any duration if HIV positive with or without production of sputum which may be blood-stained not responding to non-specific treatment (including antibiotics with no anti-TB effect i.e. avoid Rifampicin, aminoglycosides and Quinolones)
• Fever for more than 2 weeks mainly at night.
• Night sweats
• Breathlessness
• Chest pain
• Loss of appetite and loss of weight
• History of contact sometimes could be detected.

2- Any general and/or local chest manifestations.

3- A documented positive Ziehl- Neelsen (ZN) smear or culture on Lowenstein- Jensen (LJ) media for acid-fast bacilli (AFB) after appearance of symptoms.

Case definition

A Definite case of tuberculosis: Is a patient with Mycobacterium tuberculosis identified from a clinical specimen, either by smear, culture or by a newer method [7].

All studied patients were subjected to:

1) Thorough medical history stressing on general and local chest symptoms, history of diabetes mellitus (DM), receiving corticosteroid therapy and associated autoimmune disorders.
2) Full clinical examination (general and local).
3) Chest X- ray (Posteroanterior and lateral views).
4) Blood chemistry tests.

Microbiology specimen processing:

Specimen collection: early morning sputum, most probably spontaneously-produced (otherwise sputum induction by inhalation of nebulized 5-10% hypertonic saline for 20 minutes was done[11]). Sputum was collected bedside in a sterile screw-capped container with rapid delivery to the laboratory for fast processing. Specimens were taken from all of the studied patients for 3 successive days.

All sputum specimens were subdivided into three aliquots to be subjected to the followings:

- ***Zeihl Nelseen (ZN) staining for smear microscopy,*** smears were repeated on the next two days.
- ***Gene Xpert MTB/RIF PCR test*** (Cepheid, Sunnyvale, CA, USA): according to manufacture instruction, Sputum was vortexed about 1 min, sample reagent was added in a 2:1 ratio to sputum in a sterile falcon tube with manual agitation twice during a 15 minute of incubation at room temperature. Specimens were analyzed by the Xpert MTB/RIF. Two ml of this material was transferred to Xpert cartridge by a sterile disposable pipette. After operation of the test, assay's data analysis algorithm recognized a specimen as MTB positive if at least two out of the five *rpo*B probes were positive. Failure of hybridization of one or more rpoB- molecular beacons was interpreted as rifampin resistance. After finishing of PCR run, data interpretation was received through computed software.
- ***MGIT 960***, (Becton Dickinson, Sparks, MD, USA). 0.5 ml of decontaminated specimen is added to MGIT tube supplemented with 0.8 ml of Oleic Acid-Albumin-Dextrose-Catalase (OADC) beside a mixture of five antimicrobials; Amphotericin B Polymixin B, Trimethoprim, Nalidixic acid, and Azlocillin (PANTA). P-nitrobenzoic acid (PNB) test differentiated between M. tuberculosis complex and non-tubercular mycobacteria (NTM). After the positive culture flash, standard protocol for (SIRE) MGIT-DST of antituberculous agents; streptomycin, INH, RIF and ethambutol was trailed according to the manufacturer's instructions.

Statistical analysis:

Patient data, ZN staining results, Xpert MTB/RIF PCR and MGIT 960 data were tabulated and analyzed using SPSS version 19 statistical package. Data distribution were parametric.

RESULTS

Eighty patients (age ranged 21-58 years) clinically diagnosed as pulmonary TB were enrolled in this work. AFB Z-N examination was positive for in 66 out of 80, MTB detection by both Gene Xpert MTB/ RIF and MGIT 960 was positive in 74 cases.

Compared to reference lab standard test (MGIT 960), 66/74 (89.2%) were positive AFB by Z-N smear (table 2).

Twenty-one out of 74 isolates (28.4%) were rifampicin resistant by Gene Xpert MTB/RIF that was concordant with MGIT 960 SIRE results.

Table 1: Demographic data of studied patients.

Parameter	*No*	*%*
Age (years) (Mean ± SD)	35.7±13.1	
Sex:		
Male	55	74.3
Female	19	25.7
Smoking history:		
Ex- smoker	23	31
Current smoker	40	54.5
Family TB history	15	20
Presence of comorbidity	33	44.5
Use of corticosteroids	45	60.8

Table 2: Comparison between different TB diagnostic methods

	ZN smear +ve	*Gene Xpert +ve*	*MGIT 960 +ve*
Sputum sample	66 (89.2%)	74 (100%)	74 (100%)

Table 3: Comparison of processing in both BACTEC MGIT 960 and Gene Xpert MTB/RIF

Item	*Bactec MGIT 960*	*Gene Xpert MTB/RIF*
Sensitivity	100 %	100 %
Turn-around time	About 15 days	2 hours
Price	220 EGP	700 EGP
Antituberculous susceptibility	SIRE	R
No of tested specimens/ run	960	1,2,4 or 16

DISCUSSION

The scope of WHO 2016 TB guidelines update covered different areas including the effect of time to start treatment on outcomes of drug-resistant TB patient. This work was scheduled to active pulmonary TB cases as a threatening group especially with rifampicin and multidrug resistance patterns. Clinical diagnosis was based on assortment of clinical presentation and history interpretation. Mycobacteriology lab work was a mandate to confirm the final diagnosis and set as a corner stone for conclusion and start of treatment [2].

Early and rapid diagnosis helps in prevention of death and/or transmission of drug-resistant TB. Avoidance of harm by determining resistance patterns before starting treatment followed by active monitoring and controlling of drug-related toxicity during treatment [12].

Xpert MTB/RIF assay is a dual weapon, designates *M. tuberculosis*, in a semi quantitative manner coupled with RIF resistance [13].

This work showed that all smear positive specimens showed positive results on both Gene Xpert MTB/RIF and MGIT 960. However an extra 8 positive samples were detected both tools but were smear negative.

Although ZN microscopy is the available screening tool in most low-resource laboratories, false negative results are impeding of accurate diagnosis. Poor specimen quality together with a prerequisite of a skilled specialist are major constrains. Indiscrimination between drug susceptible and drug resistant strains of MTB calls for another more comprehensive test. For years, culture was the gold standard for diagnosis of MTB, but it is time-consuming continues for weeks up to months to yield results, and must be governed with laboratory facilities [14, 15].

Central TB reference laboratory is supervising the essential functions, performing quality-assured microscopy and culture. It has the first priority of being supplied by the novel modalities. Followed by regional labs, governed by financial convenience [16].

This work demonstrated that Gene Xpert MTB/RIF can detect MTB in a short turn-around time than that of MGIT 960.

The out performance of Xpert MTB/RIF detected in current work is in line with other studies, more case detection is confirmed with 49.7% relative gain by Xpert as an add-on test, recommending performing Xpert as the first investigation, to avoid extraordinary work load [17].

MGIT 960 TB system makes use of an innovative fluorometric assay via detection of O_2 utilization with high capacity [18].

After Gene Xpert endorsement by the WHO's in 2010, different researchers studied Gene Xpert's utility in different populations and countries.

A previous Egyptian study on MTB patients verified that, the sensitivity of Xpert in smear positive, culture positive was 100% and was 66.6% in smear negative, culture positive specimens with overall specificity of 100%[19]. However other resarchers disagreed, they reported a sensitivity of 65.5% [20].

Comparing with Versa TREK as the gold standard test Gene Xpert confirmed the superior sensitivity to detect MTB in smear positive specimens (100% versus 52.3% Versa TREK) in Saudi Arabia [21].

The specificity of Xpert MTB/RIF is awarded to the non–reactivity with non- tuberculous mycobacteria (NTM) even with high numbers, suggesting that the assay does not affected by NTM or cross-contamination[9].

Different studies raised the concerns of rapid drug susceptibility test (DST) methods, in particular automated MGIT 960, for detection of rifampicin resistance. Serial dilutions of MGIT960 provides further enhancement for microbiological testing, allowing the identification of the MICs of a wide range of drugs for therapy [22].

Another value added that Xpert MTB/RIF is an ultrasensitive hemi-nested PCR, does not need excessive biosafety measures, involving sample processing, permitting sample decontamination, hands-free procedure [23].Primary isolation coupled with DST of first- and second-line DST at critical drug concentrations are double-edged functions were evaluated for MGIT 960 [24].

MGIT 960 SIRE is a reference test in Central Labs, This study assessed Gene Xpert MTB/RIF DST in

comparison with MGIT 960. Current results disclosed the best sensitivity and specificity of Gene Xpert MTB/RIF regarding rifampicin resistance as results were concordant with that of MGIT. Other studies published that sensitivity and specificity of rifampin resistance in clinical specimens were designated to be 86–100% and 95–100%, respectively, with greater sensitivity in ZN-positive cases [8].

Besides, the advantage of Xpert MTB/RIF over MGIT 960 SIRE was perceived in fast identification of rifampicin cases short turn-around time of Xpert compared with prolonged time required for the reference test MGIT was 2.5 days [24].

From current operational approach (table 3), on the basis of time benefit analysis, Xpert MTB/RIF had the least turnaround time, it takes about two hours versus a couple of weeks for MGIT. On the other hand the less sensitive screening tool, ZN smear consumes time with false negative results and/or double or triple specimens, meanwhile the old standard culture on LJ takes about 4 to 8 weeks. This test is cost-effective and can be done for free for inpatients. In agreement with this study, other researchers acknowledged that, Gene Xpert testing may improve both MTB detection rate and time needed for both diagnosis and proper treatment. Thereby potentially reducing transmission of drug-resistant strains [23, 25].

CONCLUSION

To go over the main points, this study unveiled the truthful accurateness of Gene Xpert MTB/RIF assay. The best sensitivity and specificity for diagnosis of MTB and rifampicin resistance coupled with short turnaround time and total automation potentiate the utility of Gene Xpert MTB/RIF as a diagnostic tool. So, this study recommends that Gene Xpert MTB/RIF assay should be integrated in schematic approach to improve the management of pulmonary TB cases.

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