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DIAGNOSTIC ASSESSMENT OF XPERT MTB/RIF IN A SAMPLE OF *MYCOBACTERIUM TUBERCULOSIS* IN IRAQI KURDISTAN PATIENTS

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Abstract— **The Gene X pert MTB/RIF assay is a novel integrated diagnostic device for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens. We determined the performance of the MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in smear-positive and smear-negative pulmonary specimens obtained from possible tuberculosis patients.**

Study is performed in the centre of Chest & Pulmonary Diseases at Erbil city (Iraq) between November 2013 and May 2015. Three hundred fourteen Sputum samples were obtained from TB suspects. All samples were tested on Gene X pert for MTB/RIF detection after AFB microscopy. 50(15.9%) sputum samples were AFB smear positive and 263 (83.7%) were negative. In MTB/RIF assay 111 (35.3%) were MTB positive and 203 (64.6%) were negative.

The MTB/RIF assay also detected 14 RIF-resistant specimen and 97 RIF-susceptible specimens, and the results were confirmed by drug susceptibility testing. We concluded that the MTB/RIF test is a simple method, and routine staff with minimal training can use the system. It helps to avoid injudicious use of anti-tuberculosis drug.).

I. INTRODUCTION

Mycobacterium tuberculosis one of the most significant causes of death from an infectious agent. The rapid diagnosis of tuberculosis and detection of rifampin (RIF) resistance are essential for early disease management.(37)(40).And

remains a major public health problem particularly in low/middle-income countries (LMIC). More than 80% of the global TB burden and TB-related deaths were reported from 22 high-burdens LMIC in 2011 (1). With the current modest annual decline in TB incidence (2%), many countries will be unable to achieve the Stop TB Partnership goal of halving TB incidence by 2015 (2). Ambitious targets were recently set by the Partnership for the post-2015 era (3). (4)

To respond to the urgent need for simple and rapid diagnostic tools at the point of treatment in high-burden countries,(5)(34). a fully automated molecular test for tuberculosis case detection and drug resistance testing was developed through collaboration in a public–private partnership. X pert MTB/ RIF, an automated molecular test for Mycobacterium tuberculosis (MTB) and resistance to rifampin (RIF), uses hemi nested real-time polymerase chain reaction (PCR) assay to amplify an MTB specific sequence of the rpoB gene, which is probed with molecular beacons for mutations within the rifampin-resistance determining region(6), (7).Testing is carried out on the MTB/RIF test platform (Gene X pert, Cepheid), which integrates sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis , nucleic acid extraction, amplification, and amplicon detection (8) .The only manual step is the addition of a bactericidal buffer to sputum before transferring a defined volume to the cartridge. The MTB/RIF cartridge is then inserted into the Gene X pert device, which provides results within 2 hours.(9)(36).

Global TB control efforts have been severely hampered by the lack of diagnostic tests that are accurate, simple to use and can be applied at the point of clinical care. This has been further compounded by the widespread inability to test for drug resistance. The X pert® MTB/RIF assay is a rapid molecular assay that can be used close to the point of care by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 h.(10)(35)

MDR-TB essentially means that the organism is resistant to both Isoniazid (ISN) and RIF drugs which is considered most effective in treatment of tuberculosis. Patients may be infected by already drug resistant strain or the resistance may develop in erstwhile susceptible strain in the course of treatment. XDR-TB is a form of TB caused by organisms that are resistant to ISN and RIF (that is, MDR-TB) as well as any fluoroquinolone and any of the

Second-line anti-TB injectable drugs (amikacin, kanamycin or capreomycin). About 3.7% of new TB patients in the world have MDR-TB strains. Levels are much higher in those previously treated - about 20%. The frequency of MDR-TB varies substantially between countries. About 9% of MDR-TB cases also have resistance to two other classes of drugs, and hence fall into the XDR-TB category. By March 2013, eighty four countries had reported at least one XDR-TB case (11). In a nationwide survey in 2011, MDR-TB was found in 5.2 and 40.8% of patients with new and previously treated TB, respectively. These levels of drug resistance are among the highest ever documented in Africa and the Middle East. This finding presents a serious challenge for TB control (12). Worldwide, substantial percentages (˜35%) of patients with drug-susceptible TB remain undiagnosed and a staggering percentage (˜85%) of patients with MDR-TB remains undiagnosed (13). Of the people diagnosed with TB, less than 3% are tested to determine the pattern of drug resistance (14). In addition to drug resistance, another major challenge is the accurate detection of smear-negative disease which disproportionately occurs in HIV-positive people with TB (15).(16)

II. MATERIALS AND METHODS

Study is performed in the Centre of Chest & Pulmonary Diseases at Erbil city (Iraq) between November 2013 and May 2015.

Patients who presented with symptoms and signs suggestive of pulmonary tuberculosis, chest X ray showing features of pulmonary tuberculosis were included in the study. Sputum samples from these patients were sent for AFB staining as well as x pert MTB/RIF test. Early morning, deep coughed sputum specimens in sterile containers were included in this study.

The detection of Mycobacterium tuberculosis in sputum samples from suspected pulmonary tuberculosis (TB) patients has been significantly improved by the X pert MTB/RIF (Mycobacterium tuberculosis/rifampin [RIF] resistance) assay(38) .This system is an integrated diagnostic device that performs sample processing and real-time PCR analysis in a single hands-free step. The X pert MTB/RIF assay consists of two main components: (i) the X pert MTB/RIF plastic cartridge, which contains liquid sample-processing and PCR buffers and lyophilized real-time PCR reagents; and (ii) the Gene X pert instrument, which controls intra cartridge fluidics and performs real-time PCR analysis (17). The X pert MTB/RIF assay was designed to amplify a sequence of the rpoB gene specific to members of the M. tuberculosis complex and to probe for mutations within the RRDR of the rpoB gene(herb). Sequences of M. tuberculosis rpoB primers and rpoB-specific molecular beacons were modified from those described previously (18) to allow use of hemi nested PCR, to minimize cross-amplification of non tuberculosis mycobacterium (NTM) species, and to maximize mutation detection . A hemi nested molecular beacon assay to detect Bacillus globigii DNA was also included in the cartridge. This second assay tests for the presence of B. globigii spores, which are included in the X pert MTB/RIF cartridge to serve as an internal control for sample processing and PCR . Fluorescent dyes and quenchers were developed to allow all six molecular beacons to be multiplexed within the same reaction(19)(20)(21)(22)(23)

III. RESULTS & DISCUSSION

Table 1	Sputum for AFB +ve	Sputum for AFB -ve	Total samples
Gene x pert MTB +ve	50	61	111
Gene x pert MTB -ve	1	202	203
Total samples	51	263	314

Table 2	MTB +ve	MTB -ve	Total samples
RIF resistance not detected	97	203	300
RIF resistance detected	14	0	14
Total samples	111	203	314

IV. DISCUSSION

X pert MTB/RIF assay system can rapidly detect the presence of M. tuberculosis and identify the mutations most frequently associated with rifampin resistance directly from smear-negative and smear-positive clinical sputum samples. . In our study, the MTB/RIF test detected the agent in 111 of 314pulmonary specimens (35.3 % detection rate) whereas sputum for AFB was able to detect only 50 of 314pulmonary specimens (15.9 % detection rate). Out of 51 sputum smear positive cases 50 cases tuberculosis was detected by MTB/RIF test and out of 263 sputum smear negative cases 61 cases were detected.

The self-contained cartridge fluidics of the X pert MTB/RIF assay made it possible to design a hemi nested PCR assay with a sensitivity that approached culture-based diagnostics. The assay

appears to be relatively resistant to PCR inhibitors which may be present in sputum; however, PCR inhibitors may have been responsible for the one smear-positive sample from Iraqi Kurdistan that was X pert MTB/RIF negative.

Truly rapid results for drug susceptibility tests are particularly important in the management of drug-resistant tuberculosis (24). Currently available methods fall short of this promise (25,26,27,28,29,30,31,32). Most rapid nucleic acid amplification methods to detect tuberculosis require skilled technicians and dedicated space for both setup and analysis in order to prevent amplicon cross-contamination. Assay setup can also present a significant biohazard, confining work to centers with specialized biocontainment equipment. These technical requirements cause most centers to batch samples and test for tuberculosis once a day at most. The Xpert MTB/RIF assay, however, is simple and robust enough to be

performed by personnel with minimal training. Total hands-on time is less than 5 min, and results are typically available within 1 h 55 min. Each module within the GeneXpert instrument operates independently, which enables the user to test each sputum sample as it arrives in the laboratory instead of saving samples for batch processing. This important feature can potentially result in dramatically reduced turnaround times for tuberculosis detection, allowing decisions about respiratory isolation and treatment to be made in real time (33). False-positive results, often caused by carryover of amplified target, are mitigated by the use of closed cartridges that do not require any manual pipetting after the sample has been added to the cartridge. False-negative results, caused by operator errors, manufacturing defects, fluidics problems, or the presence of inhibitors in the sample, are controlled for by a multiplexed heminested PCR assay that detects a control target within B. globigii spores included within each cartridge(19)

We concluded that as compared to sputum AFB microscopy, Gene Xpert is more sensitive and specific not only for acid fast bacilli (AFB) detection but also for rifampicin (RIF) resistance. Routine staff with minimal training can use this system. It also helps to avoid injudicious use of anti-tuberculosis drugs.

The MTB/RIF test provided detection of tuberculosis and rifampin resistance directly from untreated sputum in less than 2 hours with minimal hands-on time.(39)

Multiple studies in both the industrialized and non-industrialized world showed that early identification of MDR-TB and the institution of therapy based on susceptibility in laboratory drug-resistance assays led to improved survival. The early and more accurate identification of TB cases, drug resistance and institution of appropriate therapy also removes sources of TB transmission by curing them. This combination of rapid accurate diagnosis and correct treatment is the root of all successful TB programs and public health strategies. Reducing diagnostic delay remains a high clinical and public health priority. Xpert could greatly reduce the frequency and impact of unnecessary empiric treatment,

contact investigation, and housing, providing substantial patient and programmatic benefits if used in

management decisions (38)

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