DETECTION OF RIFAMPIN RESISTANCE TUBERCULOSIS BY GENE XPERT MTB/RIF IN BASRA GOVERNORATE/ SOUTH OF IRAQ

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(**Received on Date: 12th January 2016 Date of Acceptance: 3rd March 2016**)

ABSTRACT

The current study was carried out to quantify drug resistance in tuberculous patients in Basra Province, south of Iraq using Xpert MTB/RIF.A total of 915 suspected tuberculosis patients were referred to and examined at the Advisory Clinic for Chest Diseases and Respiratory (ACCDR), the only health center that deals with this health problem in the province. Infected persons were investigated for rifampicin resistance using GeneXpert test. It has been found that about 11.25% of the examined suspected patients were tuberculous. Out of those, about 5.68% were found to be rifampicin resistant. The findings showed that the percentage of rifampicin resistance in tuberculous patients in Basrah was within the regional range of the drug resistance.

Key words: Rifampin, GeneXpert, tuberculosis, *Mycobacterium*, Basra, Iraq

No:of Figures : 3 **No:of Tables: 2** **No:of References:25**

INTRODUCTION

Tuberculosis (TB) is the second most common cause of death due to an infectious disease, and current trends suggest that TB will still be among the 10 leading causes of global disease burden in the year 2020 (Murray *et al*., 1998).Emergence of drug-resistant *Mycobacterium tuberculosis* strains is considered a real threat to achieving TB control (Zignol *et al*., 2006; WHO, 2008; Migliori *et al*., 2008).Rifampin (RIF), first introduced in 1972 as an antitubercular drug, is extremely effective against *M. tuberculosis*. It has MICs of 0.1 µg to 0.2 µg (Woodley *et al*., 1972). Resistance to RIF is increasing because of widespread application and results in selection of mutants resistant to other components of short course chemotherapy (Kochi *et al*., 1993)Resistance to RIF is associated with mutations in the gene coding for the beta subunit of RNA polymerase (*rpoB*)(Telenti *et al*., 1993; Morlock *et al*., 2000).For diagnosis of TB infections and resistance, culture remains the gold standard, but it is slow and may take up to 2 to 8 weeks (American Thoracic Society, 2005). Although smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity and a poor positive predictive value (Arzu *et al*., 2001). Thus, rapid identification, which is essential for earlier treatment initiation, improved patient outcomes, and more effective public health interventions, relies on nucleic acid amplification techniques enabling rapid implementation of treatment and minimizing of the risk of contagion. Gene Xpert MTB/RIF, an automated molecular test for *M. tuberculosis* and resistance to RIF was developed through collaboration in a public–private partnership (Hillemann *et al*., 2009; Huang *et al*., 2009; Helb *et al*., 2010). The assay is very easy to perform and almost entirely automated, requiring only two manual steps. The simplicity of the assay and its "on-demand" features make it amenable to point-of-care testing by personnel with minimal training (Banada *et al*., 2010).In Basra (south of Iraq), in the last ten years 2001-2010, the incidence rate of smear positive pulmonary tuberculosis was 19.03 per 100000 in 2001 but it declined to 8.7 per 100000 in 2010. On the other hand, the case detection rate increased from 29.74 in the year 2001 to 34.9 in the year 2010 (Figure: 1)(Habib *et al*., 2013).

The aim of the study was to genetically-based estimation of RIF resistance-pulmonary tuberculosis in Basra Governorate.

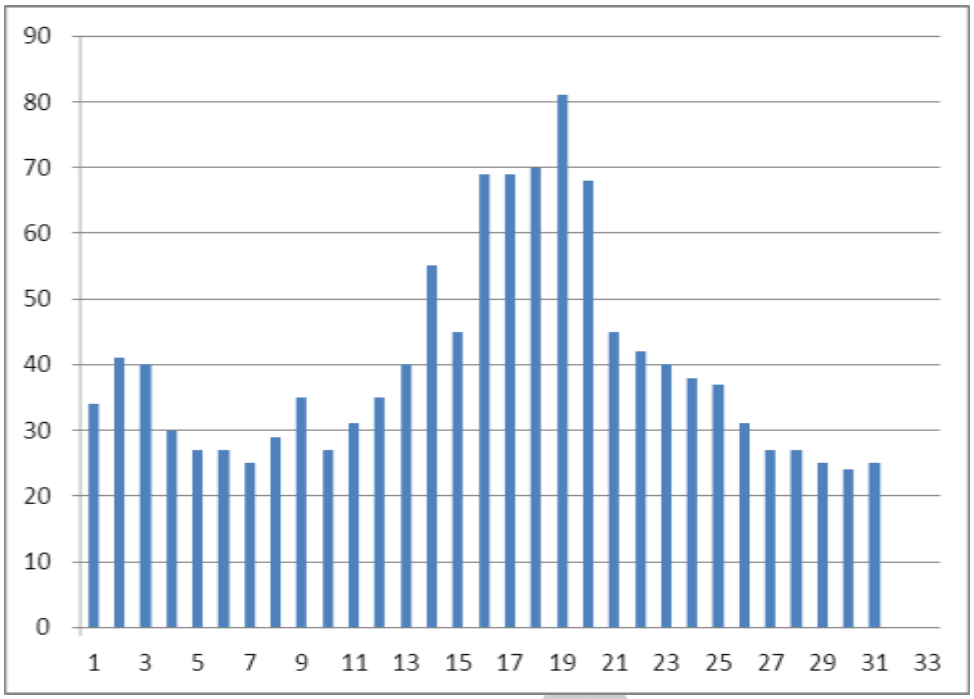

Figure (1): Annual incidence rates of tuberculosis in Basra 1980-2010(Habib *et al*., 2013.)

Materials and Methods

Study population

A total of 905 suspected have tuberculosis (new and previously treated) refers to the Advisory Clinic for Chest Diseases and Respiratory (ACCDR) from 1 January to 31 May 2014.

Sputum Smears

Samples divided into age-groups (A ten-year period of time). 10 ml sputums were collected from all persons in the early morning and directly sputum smear were examined by Ziehl Neelsen stain (ZN Stain).

Sputum cultures

Negative Ziehl Neelsen stain were decontaminated, concentrated and cultured immediately on Lowenstein-Jensen (LJ) solid medium. Briefly, A 2 to 5mL aliquot of sputum from each patient into a 50 ml falcon plastic tube. The tube was labeled with the patient's age, gender, clinical specimens were processed by 4% NaOH. Shaken for 15 min, then centrifuged for 15 min at 3000 RPM, The supernatant was removed and notarized residual by red phenol Hcl (1N), Decontaminated specimens were inoculated for growth detection and incubated at 35-37 C for 6-8 weeks (Kent and Kubica., 1985).

Identification of Rifampin resistance TB by Gene Xpert

All positive and negative samples for acid fast and LJ culture were detected of RIF-R by GeneXpert assay. The assay is simple and fast—the hand-on time is only a few minutes. Results for testing raw sputum specimens are available in 2 hours. The GeneXpert Dx System (Cepheid, Sunnyvale, CA) integrates and automates sample processing, nucleic

acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR (Raja *et al*., 2005).

Briefly, Added (2:1) sample buffer to Sediment of sputum, shake, then stand 10 min and shake then stand further 5 min. transfer 2ml to cartridge and placed in the GeneXpert machine.

Results:

All samples (915) were cultured on LJ medium after decontamination with NaOH. 103(11.25%) samples were positive for acid fast stain showing cultural, microscopical features and biochemical characterization to be identified as *M. tuberculosis*.

M. tuberculosis cells appeared as a single cells or aggregated (Figure1). The colonies of *M. tuberculosis* on LJ media were circular, rough, appears as brown, granular colonies (Figure: 2). The biochemical characterization results summarized in table (1).

A total of 915 specimens were tested for *M. tuberculosis* and RIF resistance. The smear and culture patient results tabulated below are the aggregate results for each patient compared to the aggregate Xpert MTB/RIF results for each patient positive to smear or culture. The results showed that 5.68% were found to be rifampicin resistant.

Figure (1): *M. tuberculosis* colonies on LJ medium

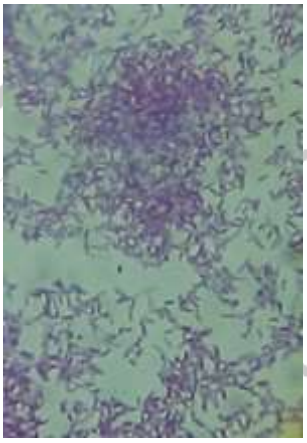

Figure (2): Microscopic feature of *M. tuberculosis* on ZN stain under immersion oil (X1000)

Table (1): Biochemical characterizations of *M. tuberculosis*

Biochemical tests	Colony features	Niacin	Nitrate reduction	Catalase	Catalase 68° C	Tween hydrolysis	Growth on 5% NaCl	Arylsulfatase	Pyrazinamidase	Urease	Tellurite reduction
M. tuberculosis	Rough	+	+	+	-	+	-	-	+/-	+	+

Table (2): Distribution of diagnosed tuberculous patients and Gene Xpert MTB/RIF results

Age(Yr)	Number of Isolates	Sex		Previous TB	Positive TB Smear AFB and LJ medium culture	Positive Gene Xpert MTB/RIF
		M	F			
10-19	102	47	55	7	6	3
20-29	156	74	82	26	20	10
30-39	201	103	98	41	25	13
40-49	167	95	72	30	19	9
50-59	147	79	68	25	17	10
60-69	104	57	47	17	10	5
70-79	38	24	14	5	6	2
Total	915	479	436	151(16.50)	103(11.25)	52

DISCUSSION

M. tuberculosis isolates (103) represented 11.25% of all collected sputum samples. As stated by the Ministry of Health of Iraq (2012), the infection rate of TB in Iraq was 45/100,000, with 13,860 new TB cases and 1140 of previously treated cases. As claimed by WHO (2011), tuberculosis deaths in Iraq reached 3,866 or 2.05%. The age adjusted mortality rate is 23.13/ 100,000.479 cases (52 %) of TB suspected patients were from males, while 436 cases from females (48 %). This may reflects the nature of work that, the males work in various fields, non sanitary, and crowded area especially in cases of poor ones. Thus, males are more exposed to infection (WHO, 2011).This result agreed with Shaker and Saleh, (2013) who found that, males were more than females in TB cases (63.2% males and 36.8% females) and also agrees with the results of Al-Jubouri (2006) and Abouzeid *et al*. (2009).

The higher appearance of *M. tuberculosis* (24.2 %) was in the age group 30-39 years (Table 2).

The GeneXpert MTB/RIF assay is a novel integrated diagnostic device for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens(Zeka *et al*., 2011). GeneXpert MTB/RIF, an automated molecular test for *M. tuberculosis* and resistance to RIF, uses heminested real-time polymerase-chain-reaction (PCR) assay to amplify an *M. tuberculosis*-specific sequence of the *rpoB* gene, which is probed with molecular beacons for mutations within the rifampin-resistance determining region(El-Hajj *et al*., 2001).The primers in the Xpert MTB/RIF assay amplify a portion of the *rpoB* gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance (El-Hajj *et al*., 2001).

In 2008, an estimated 390 000–510 000 cases of MDRTB emerged globally (WHO, 2010)In Iraq and according to the WHO (2011) tuberculosis report in the country profile for Iraq, multidrug resistance, estimated among notified cases was found to be 3.4% out of new tuberculosis cases and 21% out of the retreatment tuberculosis cases (Shaker and Saleh, 2013)

CONCLUSIONS

The study findings show that the percentage of rifampicin resistance, in tuberculous patients in Basra, lies within the regional range of the drug resistance.

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