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Molecular quantification of Rifampicin-resistance in *Mycobacterium tuberculosis*

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ABSTRACT

Aims and objectives: The current study was carried out to quantify drug resistance in tuberculous patients in Basra Province, south of Iraq.

Methods: A total of 2246 presumptive 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.

Results: It has been found that about 10% of the examined presumptive patients were tuberculous. Out of those, about 7.56% were found to be Rifampicin resistant.

Conclusions: The findings showed that the percentage, of Rifampicin resistance in tuberculous patients in Basrah was within the regional range of the drug resistance.

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Introduction

The problem of high incidence and prevalence rates of tuberculosis in Iraq is an issue of concern to the national health authorities and international parties [1], WHO [2] and UNDP [3]. Tremendous efforts have been made in Iraq to control the problem.

However, progress is very slow in controlling it [4]. It seems that one of the major obstacles, which stands against eradication of this disease, in the country, is the single/multiple drug resistance in tuberculosis patients [5]. This is not an Iraqi exclusive problem, but it is a global one.

The extent in any given country is affected by the extent of some determinants like incomplete and/or inadequate course of treatment, using a single antituberculous drug, adding a single drug to a failing regimen, non-identifying the presence of a pre-existing resistance, defaulting from treatment and/or noncompliance, following an inappropriate preventive regimen and variations in bioavailability of the drugs used in the treatment [6,7]. The first-line antituberculosis drugs include Isoniazid, Rifampicin, Pyrazinamide, ethambutol and Streptomycin [8].

There are two types of antituberculous drug resistance, mono- and multi- drug resistance [8]. Internationally, studies, using different methodologies and conducted in different times, gave different figures for this resistance. In Burundi, it was reported that from new cases about 16.1% showed resistance to any drug, 6.3% to isoniazid, 2.0% to Rifampicin (1.4% multidrug-resistant tuberculosis), 13.3% to streptomycin and 1.6% to ethambutol. From previously treated cases, the prevalence of resistance was 30%, 19%, 15% (12% multidrug-resistant tuberculosis), 25% and 6%, respectively [9]. In China, about 26.3% of the isolates showed resistance to at least one drug. The overall prevalence of multidrug-resistant tuberculosis was 6.8% (3.0% of newly diagnosed patients and 22.0% of previously treated cases) [10]. In the Eastern Mediterranean Region, Surucuoglu et al. from Turkey found that any-drug resistance and multidrug resistance were 21.1% and 7.3% respectively [11]. Tabarsi et al. from Iran reported that out of the study tuberculous patients, 43% were 'any-drug resistant' cases. About 32.3% were mono-drug and 6.1% were multi drug resistant. Previous antituberculosis medication was significantly associated with any-drug resistance. Streptomycin showed the highest resistance rate (27%) followed by isoniazid (20%), pyrazinamide (9.8%), Rifampicin (9.2%) and ethambutol (3%) [12]. Finally, a study in Saudi Arabia found reported that 12.9% of the patients were with any-drug resistance (from them about 6% with resistance to Rifampicin) and about 2.6% patients with multidrug resistance tuberculosis [13].

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In Iraq and according to the 2011 WHO 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 [14].

It was reported that detecting Rifampicin resistance prevalence rate among tuberculous patients can be considered as a surrogate marker of tuberculosis multidrug resistance [15].

The current study was carried out to quantify, for the first time in Basra Province, the resistance to Rifampicin in tuberculous patients using the GeneXpert test.

Methods

A descriptive cross-sectional study was conducted to achieve the objective of the study. A total of 2246 presumptive tuberculosis patients, who were referred during 2013 to the Advisory Clinic for Chest Diseases and Respiratory (ACCDR), the only health center that deals with such health problem in the Basra province, south of Iraq, were examined. They were referred by the governmental primary healthcare centers and hospitals and private clinics from all over the Province. At the ACCDR, the patients were re-examined clinically, and chest X-ray, microscopic examination for acid fast bacilli, and sputum culture (concentrated and cultured immediately on Lowenstein-Jensen solid medium) investigations were done to diagnose the disease.

Out of the diagnosed as tuberculous patients, the previously treated ones (relapsed, failed-treatment, and defaulted from treatment cases) (59) and sputum-smear positive new cases (166) were tested for Rifampicin resistance by using GeneXpert.

Results

The most frequent age group of tuberculosis presumptive patients attended the ACCDR due to referral, who were examined, during 2013 was 35–44 years (Table 1).

Out of the numbers above, the number of patients, who were diagnosed as tuberculous using chest X-ray, detection of acid fast bacilli, and culture, was 586 patients. Table 2 shows the distribution of diagnosed tuberculous patients according to age groups.

Table 1
Sputum-smears examined during 2013 from TB presumptive patients.

Age group (years)	Number ^a	Percentage
0–4	0	0.00
5–14	104	4.63
15–24	305	13.59
25–34	419	18.66
35–44	451	20.09
45–54	354	15.77
55–64	334	14.88
65 +	278	12.38
Total	2245	100.00

^a There was one case missed.

Table 2
Distribution of diagnosed tuberculous patients according to age groups.

Age group (years)	Number	Percentage
5–14	45	7.68
15–24	101	17.24
25–34	95	16.21
35–44	119	20.31
45–54	83	14.16
55–64	78	13.31
65 +	65	11.09
Total	586	100.00

Table 3
Distribution of the patients diagnosed having tuberculosis according to category.

Category	Number	Percentage
Previously treated	59	10.07
New cases:	527	89.93
Sputum-smear positive	169	
Others ^a	358	
Total	586	100

^a Others = sputum-smear negative, extra-pulmonary tuberculosis, and not-done sputum-smear examination.

Table 4
Distribution of patients with Rifampicin-resistant tuberculosis according to age groups.

Age group (years)	Number
5–14	0
15–24	0
25–34	4
35–44	6
45–54	4
55–64	2
65 +	1
Total	17

The categorization of those patients is shown in Table 3, when previously treated patients constituted about 10.07% out of the total number of the patients.

When sputum samples, from the 586 tuberculous patients, were tested by the GeneXpert, 3 samples from the 169 sputum-smear positive new cases samples were found to be negative. Out of the 59 previously treated patients and 166 sputum-smear positive new cases, who were tested for Rifampicin resistance using GeneXpert, 17 patients (7.56%) were found to have resistant tuberculosis. Out of those, 10 were from the previously treated category (16.95%) and 7 were from the sputum-smear positive new cases (4.22%). Table 4 shows the distribution of those patients according to age groups. Twelve of the patients were males and 5 were females.

Discussion

Iraqi Ministry of Health have put a target for tuberculosis case detection rate at 45/100,000 population [16]. As far as, Basra total population was estimated to be 2,664,814 during 2013 [17], then the expected tuberculosis cases detected was about 1199 patients. The diagnosed number reported in this study, 586, was about half the expected figure. This can be attributed either to insufficient screening programme or to the falsely high estimated case detection target.

Although, the people in the age group 35–44 years among the Iraq population constitute about 12.66% [18] only, tuberculous patients in the same age group showed the highest frequency among all tuberculous patients diagnosed in the study (20.31%). This age group was higher than the age group of highest frequency found in another Iraqi study conducted in Baghdad (26–35 years) [5]. However, this is not the case in the more developed countries, where cases are detected more among older age groups, above 65 years [19,20]. This is most probably because, in Iraq, people with tuberculosis risk factors in these older age groups die, due to other reasons, before acquiring tuberculosis infection or being diagnosed after being infected. In the more developed countries elderly people show considerable levels of morbidity but lower level of mortality in comparison to the situation Iraq.

The percentages of old (10.07%) and new (89.93%) cases reported in the current study slightly differ from those reported by Al-Obaidy (21.1% and 78.9% respectively) [5] and by Surucuoglu et al. (19.03 and 80.97%) [11].

The 3 samples from the 169 sputum-smear positive new cases samples that were found to be negative may refer to a false diagnosis or bovine or other atypical tuberculosis.

Resistance prevalence rate to Rifampicin in both old and sputum-smear positive new cases documented in this study (7.56%) was relatively close to what have been reported by Surucuoglu et al. (9%) [11], Tabarsi et al. (9.2%) [12], and Singla et al. (6%) [13]. However, this rate differs to a considerable extent from the prevalence rate reported by Al-Obaidy in Baghdad (14.3%) [5]. This difference needs more research in Iraq to determine whether this difference is a real one or due to differences in methodology.

It has been stated that the presence of a history of antituberculosis treatment was the strongest predictor of drug resistance [21]. The findings of this study agree this statement, when the resistance prevalence rate in the previously treated category was 16.95%, while in the new cases category it 4.22%. Both findings were similar to what have been reported by Sharma and Mohan [9] and Surucuoglu et al. [11].

An important point needs to be highlighted here; that is the prevalence of resistance, found in the current study, represents the existence of such a resistance among the patients diagnosed from those who attended the ACCDR. Considering the targeted case detection rate and the possibility of insufficiency of the screening programme because of the considerable difference between the target and the actual number of patients diagnosed, there is a likelihood that the rates of Rifampicin resistance detected are inclusion biased.

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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