Pattern of mycobacterium tuberculosis drug resistance in previously treated cases in Iraq

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Abstract

Background: The resistance of certain *Mycobacterium tuberculosis* strains to anti-tuberculosis drugs is not a new phenomenon. It is man-made amplification of natural phenomenon.

Objectives: 1- To provide scientifically based information on the burden of Mycobacterium tuberculosis drug resistance in Iraq. 2- To compare the pattern of this resistance in Iraq with that in the other countries.

Methods: A total 411 patients with pulmonary tuberculosis who have already received at least one month of anti-tuberculosis therapy were selected. Sputum cultures and drug sensitivity tests for Mycobacterium tuberculosis were arranged.

Results: Resistance to rifampicin, isoniazid, streptomycin and ethambutol was noted in 52 (24.4%), 22 (10.3%), 21 (9.9%) and 8 (3.8%) of isolates respectively. Multidrug and four-drug resistance was found in 52 (24.4%) and 24 (11.3%) respectively. Rifampicin resistance in any form was noted in 146 (68.5%).

Conclusion: The magnitude of *Mycobacterium tuberculosis* drug resistance in Iraq found to be relatively high.

Key wards: tuberculosis; antituberculous drugs resistance; multidrug resistance; drug resistance

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Introduction

The emergence of resistance to antimicrobials is a natural biological occurrence. Drug resistance can be divided to: **Drug resistance among new cases (primary resistance)** referred to presence of resistant isolates of tuberculosis (TB) in patients who deny or having no evidence of, prior anti-TB treatment (for as much as 1 month), **drug resistance among previously treated cases (acquired resistance)** which is the presence of resistant isolates of tuberculosis in patients who, in response to direct questioning, admit having been treated for tuberculosis for period of 1 month or more, and **combined prevalence of drug resistance** which is the prevalence of resistance in the population surveyed regardless of prior treatment[1]. *Polyresistance* means resistance to more than one drug other than multidrug resistance.

In 1995, WHO estimated that 50 million people were infected with drug-resistant strains of *Mycobacterium tuberculosis* [2]. In 1997, the World Health Organization (WHO), the International Union against Tuberculosis and Lung disease (IUATLD) and several partners worldwide release the first report of Global Project on Anti-tuberculosis Drug Resistance Surveillance (DRS). This report presented data from 35 geographical settings (surveyed between 1994 and 1996) using standard epidemiological and laboratory guidelines. These data covered 16% of the world notified TB cases.

Drug resistance is less likely to occur in directly observed therapy (DOT) [3]. The survey in Islamic

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Republic of Iran demonstrated high prevalence of multidrug resistant-TB (5%) and any rifampicin (R) resistance (50%). Drug resistance is not a problem in Oman, nor is it in Morocco. Iraq was not included in this project.

The majority of isoniazid (H) resistant strains show mutations of the *katG* gene (50%) followed by mutation locus resistance in *inh*A (25%) of total H resistance. Mutation loci for rifampicin resistance is rpoB (97%), for streptomycin (S) is rpsL (60%), for ethambutol (E) is embAB (50%) and for pyrizinamide (Z) is pcrA with unknown revalence[4,5].

Methods

A total of 411 randomly selected cluster of patients included. All have pulmonary TB and had already received at least one month of anti-TB therapy. The study was conducted from February 2005 to August 2006. All patients selected were sputum positive isolates of *M. tuberculosis* (using standard Zeihl-Nelson stain method). Culture and sensitivity then arranged for patient's sputum samples.

Setting: Institute of tuberculosis and chest disease in Baghdad, receiving patients from all hospitals all over the country, and the first treating facility for a large population in Baghdad. It is the only facility that has performs culture and drug susceptibility testing for *Mycobacterium tuberculosis* (MTB) in Iraq.

Bacteriological methods

Löwenstein-Jensein (L-J) culture medium was used for culturing sputum. Identification of the strains was based on the niacin production test, the nitrate reduction test, the amniobenzoic acid (500mg/l) and the thiophene carboxylic acid (2mg/l) resistance test. Species other than the pathogenic species of MTB complex were excluded from analysis, e.g. rapid grower species that grow within three days.

Cultures that yield MTB then subjected to drug sensitivity tests. Drug sensitivity test was performed using the proportion method on L-J medium.

Using the proportion method, resistance was expressed as percentage of colonies that grew on critical concentrations of the drugs which were prepared as follows:

H: 0.2 µg/ml, R: 40 µg/ml, E: 2 µg/ml and S: 4 µg/ml. The criterion for resistance to a particular drug was growth of 1% of the population on medium containing the critical concentration. The results of the tests recorded on standardized laboratory forms.

Radiometric BACTEC MGIT 960 (Becton Dickinson) method, started to be in work since 2002 in Iraq, used for both mycobacterial detection and drug susceptibility testing. Using this method, resistance was expressed as growth concentration in each tube containing the following concentrations (compared to growth control): H: 0.1 µg/ml, R: 1 µg/ml, E: 5 µg/ml and S: 1 µg/ml.

Screening for the human immune deficiency virus (HIV) was not part of this study.

Statistical analysis

SPSS ® version 10.0.5 for Windows to analyze the data. The Student t-test was used to calculate continuous variables. A *P* value of 0.05 was considered significant.

Results

Three hundred-eleven (73%) were males and 100 (24.3%) were females. The median age of the patients was 34 years. Mean age = 35.4 ± 10.4 years with a male: female ratio of 3:1. The age group most commonly encountered in

this study range between 25 to 44 years: 206 (50%) males and 70 (17%) females. The median age of patients **with resistant** MTB was 34 years (range 20-65 years) for males and for females was 35 (age range 20-54 years), while for those **with sensitive** isolates the median age was 34 years for males(age range 20-67 years), and for females it was 35.5 years (age range 21-68 years) (Table 1).

One hundred eighty-nine (48.2%) isolates were sensitive to H, R, S and E, and 213 (51.8%) were resistant to at least one of the four first-line agents. Multidrug resistance was found in 52 (12.6%) isolates. Four-drug resistance was highest among the MDR group, noted in 24 (5.8%). Any drug resistance noted in 213 (51.8%) (Table 2) (Figure 1and 2).

Monoresistance was noted in 103 (25.1%). As monoresitance, resistance to R was the highest, noted in 52 (12.6%) isolates; H resistance was the second most common, found in 22 (5.3%), S resistance was noted in 21 (1.9%) and E resistance was the lowest, in 8 (1.9%) isolates. Combined resistance of S with R and in other hand S with H was found to be higher than other types of polyresistance, 20 (4.9%) and 10 (2.4%) respectively. Rifampicin resistance, in any form, exceed any other type of resistance where it was noted in 146 (35.5%), followed by H in 103 (25.1%), S in 93 patients (22.8%) and E in 47 patients (11.4%) (Table 2) (Figure 2).

When compared to other geographical settings, drug resistance in Iraq outweigh that in Saudi Arabia in (a) *any resistance*: (51.8% compared to 22%), (b) *1, 2 and 3 drug(s) resistance*: (26%, 14% and 7% compared to 6%, 6% and 2%) and (c) *MDR*: (12.6% compared to 2%). While four drug resistance in Iraq is lower than that in Saudi Arabia: 6% compared to 8%.

In comparison to Islamic Republic of Iran, overall resistance in Iraq was *lower*, (51.8% compared to 57.1%), as well as 4-drugs: (11.3% compared to 28.6%) and MDR (12.6% compared to 48.2%) respectively, while resistance to 1, 2, and 3 drugs were *higher*, (48.45, 25.4%, 12.2% compared to 7.1%, 14.3%, 7.2%) respectively (Table 3) (Figure 3).

Table 1: Characteristics of the study sample

Sensitivity	Gender	Mean age	Median age	Age range
Sensitive	Male	34.9 $\pm$ 10.5	34	20 – 67
	Female	37.2 $\pm$ 10.9	35.5	21 – 68
Resistant	Male	35.3 $\pm$ 10.6	34	20 – 65
	Female	35.5 + 9.5	35	20 – 54

Table 2: Patterns of anti-tuberculosis drug resistance (in previously treated cases)

	Number	%
Total tested	411	100
Fully sensitive	198	48.2
Any resistance	213	51.8
Mono-resistance	103	25.1
H*	22	5.3
R**	52	12.6
E***	8	1.9
S****	21	5.1
H + R resistance	52	12.6
HR	15	3.6
HRE	9	2.2
HRS	14	3.4
HRSE	24	5.8
H + other resistance	12	2.9
HE	2	0.5
HS	10	2.4
HES	0	0.0
R + other resistance	28	6.8
RS	20	4.9
RE	5	1.2
RES	3	0.7
Other multi-resistance	2	0.5
ES	2	0.5
Any H resistance	103	25.1
Any R resistance	146	35.5

*H= isoniazid, **R= rifampicin, ***E= ethambutol, ****S= sreptomycin

Table 3: Pattern of drug resistance to each drug among previously treated cases in three geographical settings (figures in percentage)

Country	Year	No. of patients tested	Overall		Resistance to:				Poly-resistance	
			Susceptible %	Resistant %	1Drug %	2Drugs %	3Drugs %	4Drugs %	Any %	Multidrug resistance %
Iraq	2006	411	48.2	51.8	48.4	25.4	12.2	11.3	14.2	12.6
Iran	1998	666	42.9	57.1	7.1	14.3	7.2	28.6	50	48.2
Saudi Arabia	2004	764	91.5	22	6	6	2	8	2	2

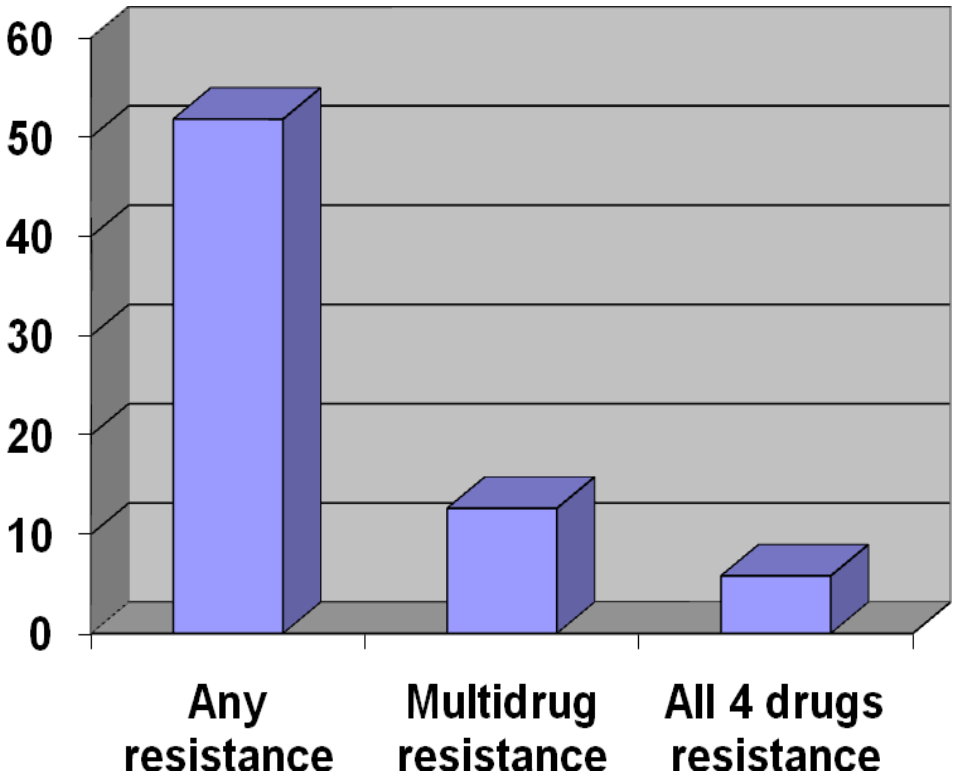

Figure 1: Drug resistance indicators among previously treated cases

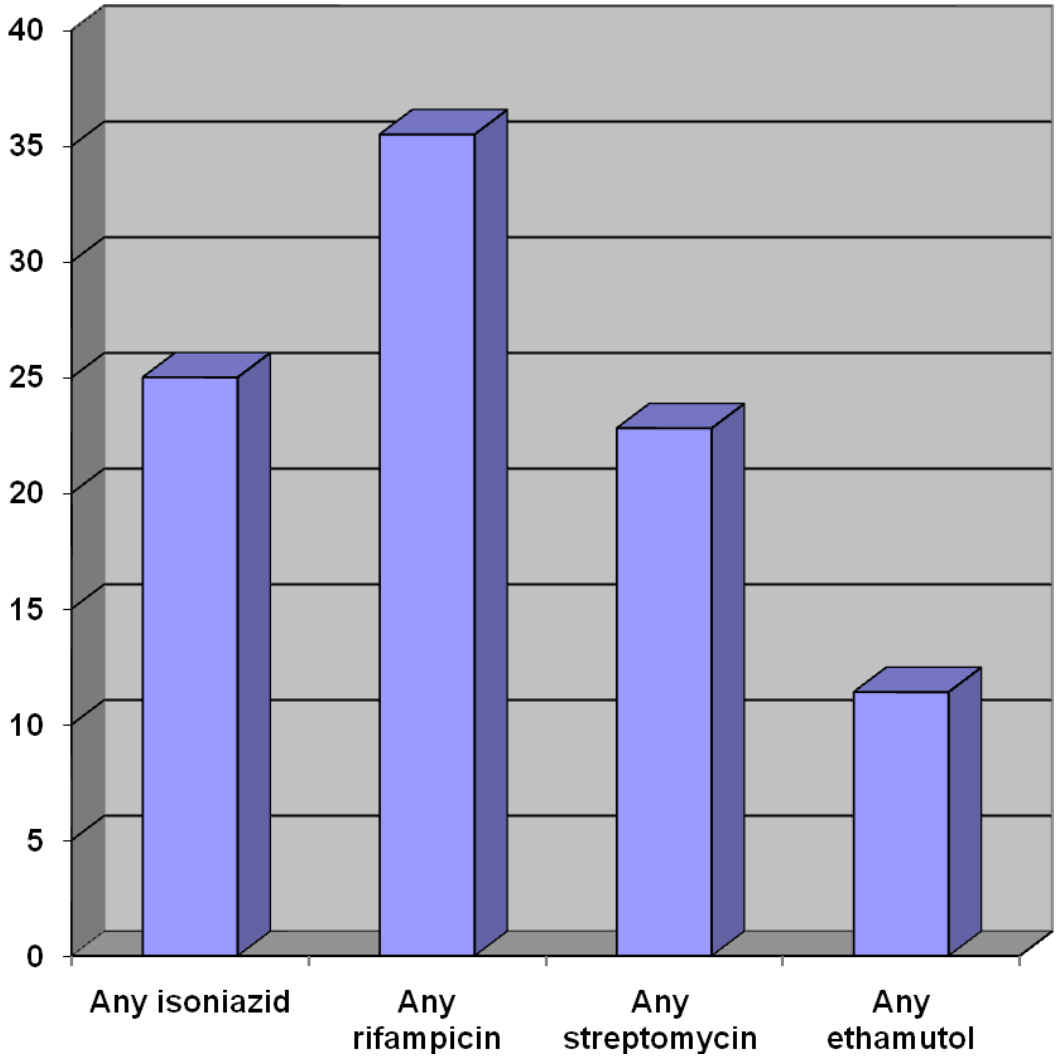

Figure 2: Prevalence of any drug resistance among previously treated cases according to specific drugs

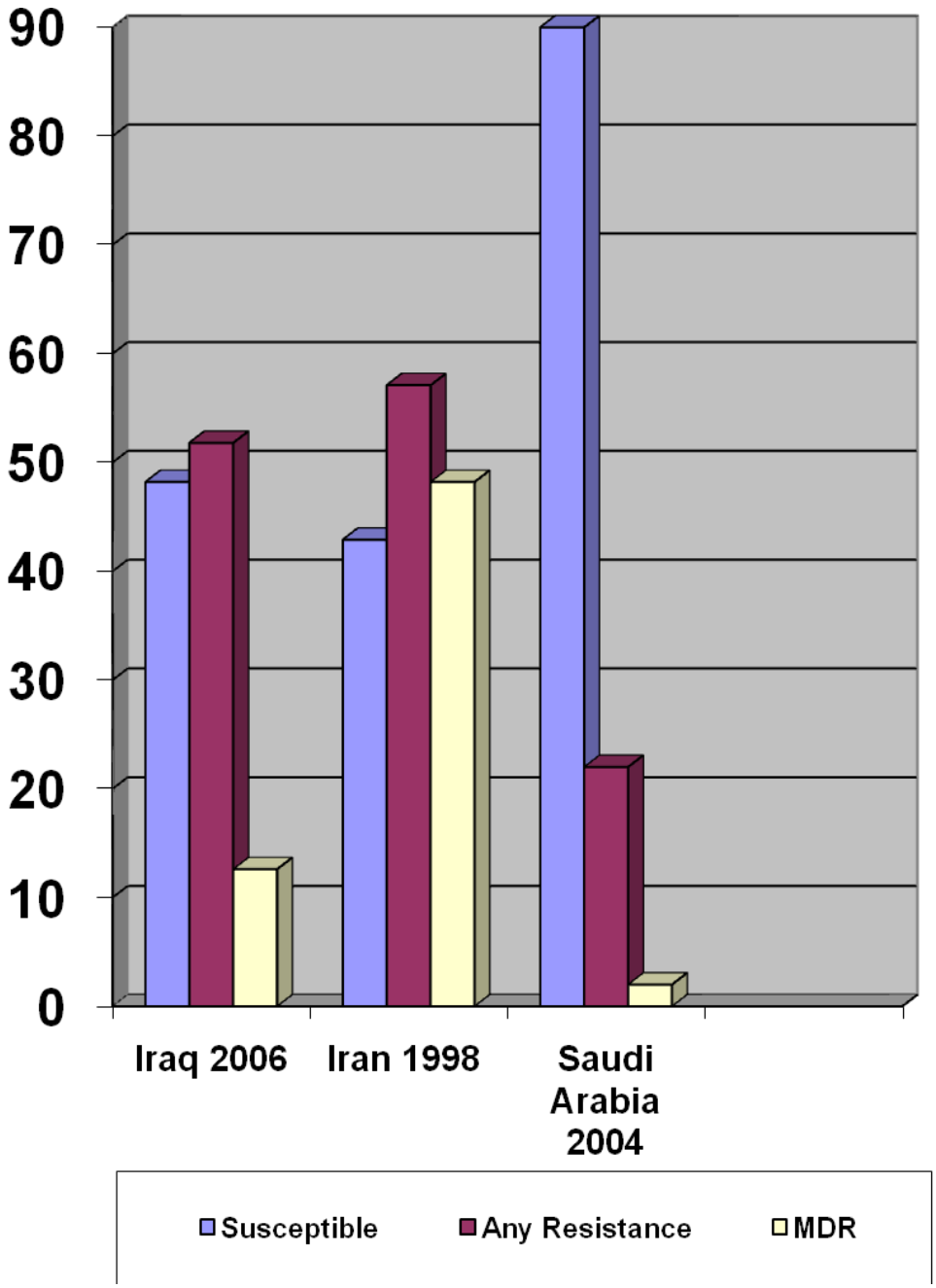

Figure 3: Pattern of multidrug resistance, any drug resistance and susceptible cases in three geographical settings

Discussion

Reports of drug resistance of *M. tuberculosis* in Iraq are limited. The prevalence of drug resistance to one drug (**monoresistance**) was 25.1%, while that of resistance to **all four drugs** was 58%. Monoresistance is higher and four-drug resistance is lower than that in Islamic republic of Iran, and both are higher than that in Saudi Arabia. In the Global Project the median prevalence of drug resistance to one drug was 11.3, and the median prevalence of resistance to all

four drugs was 1.8 [6].

Any drug resistance among previously treated cases found to be 51.8% which is higher than that in Saudi Arabia (22%) [7] and lower than that in Islamic Republic of Iran (57.1%). This type of resistance falls in the median value when compared to other countries in Global Project which is ranged from 0% in Finland to 93.8% in Uruguay with a median prevalence of 23.3 %[8].

Increases in prevalence of *any resistance* may reflect an environment that favors the acquisition of additional resistance and can lead to future increases in MDR [1].

High number of drug-resistance among previously treated cases seen in this study may be related to high number of resistant strains circulating the in the community due to very high pool of previously treated cases.

MDR-TB among previously treated cases was 12.6%. It is ranged from 0% in 4 geographical settings in Global Project, to 48.2% in Islamic republic of Iran. The median prevalence in Global Project was 9.3%[9]. In Saudi Arabia it is 14 %[7]. The most powerful predictor of MDR-TB was a history of past treatment, which was usually accompanied by inadequate drug regimen [10]

Monoresistance to rifampicin (R) was (12.6%) outweighing other types of monoresistance (H, E, and S), but is lower than that found Egypt (21.7%)[1] and it was not recorded in Islamic Republic of Iran nor in Saudi Arabia [7,8]. In addition, relatively high prevalence of **any rifampicin resistance** (35.5%), the most powerful anti-tuberculosis drug and a key determinant of MDR-TB, was reported. It is higher than that recorded in Egypt (7%)[1] and Saudi Arabia (14%)[7] but lower than that in Islamic Republic of Iran (50%)[8]

Rifampicin might be used in Iraq, unexpectedly, to treat other types of infections, like brucellosis and geneto-urinary infections as well as incorrectly prescribed or taken by patients - singly or inappropriately combined - with other anti-TB; therefore, high level of resistance to this very important anti-TB drug could be produced.

Any resistance to streptomycin (S) was observed in 22.8%, lower than the value found in Egypt (23.6%)[1] and Iran (39.3%)[8] but higher than Saudi (12%)[7].

Any resistance to ethambutol (E) was noted in 11.4%, which is more than Saudi 10%[7], but lower than Iran 32.1%[8], and Egypt (30.9%)[1].

Polyresistance was noted in 23% of isolates, which is higher than that found in Saudi (1%) [7], and lower than that found in Iran (50%) [8].

When compared to other geographical settings, drug resistance in Iraq outweighs that in Saudi regarding *any resistance* (51.8 compared to 22%), while it is lower than that in Iran (57.1%). Resistance to *one drug* was 25.1 which is more than that in Iran and Saudi (7.1 and 6 respectively). *Tow and three drug* resistance were found to be 13.1 and 6.3%. Both percents are higher than that found Saudi (6 and 2%) and lower than Iran (6% and 14.3 respectively).

In comparison to Islamic Republic of Iran, *overall resistance* in Iraq was lower (51.8% compared to 57.1%), as well as *4-drug resistance* (5.8% compared to 28.6%) and *MDR* (12.6% compared to 48.2%) while resistance *to 1, 2, 3 drug(s)* were higher, (48.45, 25.4%, 12.2% compared to 7.1%, 14.3%, 7.1%), (Table 3) (Figure 3).

From total cases sampled, cases

with *any resistance* exceed those who were sensitive by about 3%. This may be regarded as a **bias** related to the drawback of this study in the way of collecting samples, where patient with TB suspected of having some problem (resistance or other complications), were referred to the single institute from different country cities, therefore, the high number of resistance cases found.

Many factors acting locally, affect TB control and drug resistance prevalence. These include non-use of DOT regimen in Iraq, effect of war and other social and economic limitations.

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