The Pattern of Drug Resistance in Iraqi Pulmonary Tuberculosis Patients Referred to the Specialized Center for Chest and Respiratory Disease

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ABSTRACT:

BACKGROUND:

Tuberculosis control in the world today must face the challenge posed by the global spread of Mycobacterium tuberculosis strains that are resistant to standard anti-TB drugs.

OBJECTIVE:

This study was done to identify the drug resistance to the first line antituberculosis drugs in Iraqi Patients.

METHODS:

This prospective study conducted in National Center for Tuberculosis and Chest Diseases in Baghdad from January 2012 to August 2012.For study the demographic characters and drug sensitivity test (DST) in a total number of 155 case of sputum smear positive pulmonary tuberculosis (97 old and58 New) For each patient 3 consecutive sputum samples were taken for direct smear microscopy and cultured to test drug resistance for First Line anti-TB drugs (INH, Rifampicin ,Ethambutol and pyrazinamide).

RESULTS:

In this study we found ethambutol resistant in 20(34.5%)of samples in the new group and in 49(50.5%) of samples in old group. On the other hand,our study showed that resistance to rifampicin in the new group was 58.6% while in the old group was 75.3%. In regard to the INH drug resistance it seen in 72.4% of samples in new group and in 83.5% of samples in old group**.**This study showed that reistance to streptomycin present in 44.8% of new group samples and in 55.7% of old group samples .The pattern of drug resistance according to patient group show high frequency of polyresistance 82 cases in both patient group(new and old) and low frequency in other group of resistance 14 cases,and MDR type of resistance in total of 17 cases .

CONCLUSION:

The study showed 11%(17/155) of patients with pulmonary tuberculosis in this sample of patients had multidrug resistance MDR tuberculosis .

KEY WORDS:drug resistance ,tuberculosis .

INTRODUCTION:

Tuberculosis (TB) control in the world today must face the challenge posed by the global spread of Mycobacterium tuberculosis strains that are resistant to standard anti-TB drugs [(1)]. Multidrug-resistant (MDR) tuberculosis (TB), which is defined as infection with a Mycobacterium tuberculosis complex isolate that is resistant to at least isoniazid (INH) and rifampin (RIF), is a public health concern that threatens the success of global TB control programs[(2)]. It is estimated that ~3% of incident new TB cases in the world have multidrug-resistant TB (MDR-TB) [(3)].

Treatment for MDR-TB patients requires the use of second-line drugs for at least 18 to 24 months [(4)]. Therefore, the WHO has expressed concern over the emergence of virulent drug-resistant strains of M. tuberculosis and is calling for stronger measures to prevent XDR-. Around 440,000 MDR-TB cases are estimated to emerge annually among new and retreated TB patients. The frequency of MDR-TB varies according to region and is much higher

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among previously treated patients. Amongst the vast majority of MDR-TB patients, very little is known about their access to quality care. Treatment of MDR-TB is complex and uses toxic drugs that must be administered for a longer duration than for drug-susceptible TB patients, with a lower likelihood of treatment success [5].

Understanding the mechanisms of mycobacterial resistance to the anti-tuberculosis drugs not only enables the development of more rapid molecular diagnostic tests and furnishes implications for designing new anti-tuberculosis drugs, it also helps to implement measures to prevent the development of such resistance. Drug-resistant TB is not a recent phenomenon. M. tuberculosis strains that were resistant to streptomycin (SM) appeared soon after the introduction of the drug for treatment of TB in 1944. Genetic resistance to an anti-tuberculosis drug is due to spontaneous chromosomal mutations at a frequency of 10−6 to 10−8 mycobacterial replications. Mobile genetic elements such as plasmids and transposons, which are known to mediate drug resistance in various bacterial species, do not do so in M. tuberculosis. Because such mutations resulting in drug resistance are unlinked, the probability of developing bacillary resistance to three drugs used simultaneously becomes 10−18 to 10−20. In theory, the chance of drug resistance is thus virtually non-existent when three effective drugs are used in combination for TB treatment [6].

Amplification of the afore-mentioned genetic mutation through human error results in clinically drug resistant TB. These include ‘monotherapy’ due to irregular drug supply, inappropriate doctor prescription and, most importantly, poor patient adherence to treatment [7]. Subsequent transmission of resistant M. tuberculosis strains from the index patient to others aggravates the problem . The MDR/XDR phenotype is caused by sequential accumulation of mutations in different genes involved in individual drug resistance . Although the definitions of ‘acquired’ and ‘primary’ drug resistance are conceptually relatively clear, in reality they are often subject to misclassification when previous treatment cannot be readily ascertained. The term ‘initial’ drug resistance is thus often preferred to ‘primary’ drug resistance to include ‘unknown’ or ‘undisclosed’ acquired drug resistance. The matter is currently further simplified by categorizing drug resistance in new cases and previously treated cases of TB [2]. The latter refers to cases with treatment lasting for at least 1 month.

AIM OF THE STUDY:

1.To study the patterns of drug resistance to the first line antituberculosis drugs .

2. Evaluation of new reveral system for sending patients from periphral laboratories To NRL.

PATIENTS AND METHODS:

This prospective study conducted in National Center for Tuberculosis and Chest Diseases in Baghdad from January 2012 to August 2012.For study the demographic characters (Age ,Sex ,residency) and drug sensitivity test (DST) in a total number of 155 cases of sputum smear positive (SS+) pulmonary tuberculosis (106 males and 49 females ,Age range 13-70 year). A patient was eligible for inclusion in this study if registered as sputum smear – positive case (new or previously treated). During this period ,a 97 cases (69 males,28 females) were referred to the National Research Lab.(NRL) from Baghdad and all Iraqi governorates for DST so these cases were labeled as (old group) who had received antituberculosis treatment previously for at least 4 weeks which include three treatment categories of patients (Failure ,Relapse ,Defaulter).The results of this group were compared with that of control group which includes 58 new patients who never receive antituberculosis treatment previously(New group: which include 37 males and 21 females). For each patient 3 consecutive sputum samples were taken for direct smear microscopy and exposed for concentration, digestion and decontamination by petroff method and then cultured on Lowenstien-Jensen solid media, and positive culture was taken for indirect proportional method for DST to tested drug resistance for First Line anti-TB drugs (INH, Rifampicin ,Ethambutol and pyrazinamide). Resistance is expressed as the percentage of colonies that grow on critical concentration of the substance, i.e. 0.2 mg / L for isoniazid, 2 mg /L for ethambutol, 40 mg /L for rifampicin if L –J medium has been used. Inclusion criteria for new cases include pulmonary (extrapulmonary excluded), sputum smear positive (SS+) and not had history of DST testing and or consider as drug resistance on clinical base. For Old cases, inclusion criteria include pulmonary, sputum smear positive (SS+) and had history of DST testing and or consider as drug resistance on clinical base.

The old referal system for evaluation of drug resistant for antituberculosis drugs include sending the patients themself from TB centers in differnt governerates of Iraq to the National TB Center . In

this study we evaluate the new referal system which started in January 2012 and include sending sputum samples of patients suspected to have drug resistance on clinical bases (rather than pateints themselves) by authorized employer.

RESULTS:

Table 1: The mean of age according to patient groups.

	Group	n	Mean years	Std. Deviation
Age	new	58	36.0690	±12.41796
	old	97	37.2062	±12.88647

P=0.59

The results of mean age in new group was 36.06 years with std ± 12.41 and for old group was 37.20 years with std 12.88±(there was no statistical difference P=0.59).

Table 2: Frequency distribution of gender according to patient groups

			group		Total
			new	old	
gender	male	n(%)	37(63.8)	69(71.1)	106(68.4)
	female	n(%)	21(36.2)	28(28.9)	49(31.6)
Total		n(%)	58(100.0)	97(100.0)	155(100.0)

The frequency distribution of gender in this study according to patient group showed no significance difference with P=0.219 with 37 male and 21 female in new group and 69 male and 28 female in old group.

Table 3: The results of patients residency* according to patient groups

			group		Total
			new	old	
Address	Baghdad	n(%)	33(63.8)	47(48.5%)	80(51.6%)
	Euphrates	n(%)	4(6.9%)	13(13.4%)	17(11.0%)
	Middle	n(%)	7(12.1%)	8(8.2%)	15(9.7%)
	South	n(%)	6(10.3%)	14(14.4%)	20(12.9)
	Kurdistan	n(%)	8(13.8%)	15(15.5%)	23(14.8)
Total		n(%)	58(100.0)	97(100.0%)	155(100.0%)

*Euphrates: Al-Muthannā ,Al-Qādisiyyah ,Bābil,Karbalā' ,An-Najaf
Middle: Salāh ad-Dīn ,Diyālā ,Al-Anbar ,Nīnawā .
South: Wāsit ,Maysān ,Al-Basrah ,Dhī Qār .
Kurdistan: Duhok,Arbīl, As-Sulaymāniyyah

The residency of TB patients showed high frequency in Bagdad with 80 patients respectively and the Middle area showed lowest frequencies of cases with 15 cases (no significance difference P=0.5).

Table 4 : DST for anti-TB drugs according to patient groups.

Anti-TB drugs		New group	Old group	P-value
Ethambutol	sensitive	38(65.5%)	48(49.5%)	0.037
	resistance	20(34.5%)	49(50.5%)	
Rifampcin	sensitive	24(41.4)	24(24.7)	0.024
	resistance	34(58.6)	73(75.3).	
INH	sensitve	16(27.6%)	16(16.5%)	0.075
	resistance	42(72.4%)	81(83.5%)	
Streptomycin	sensitive	32(55.2)	43(44.3)	0.127
	resistance	26(44.8%)	54(55.7%)	

The DST results of ethambutol drug in new group were sensitive in 38 cases and resistance in 20 cases. In old group the results were sensitive in 48 cases and in 49 cases were resistant, the resistance to ethambutol was more in the old group (49 cases) than in new group (20 cases) .There was a significant statistical difference (P- value =0.037).
The testing of DST of rifampicin drug in new group was sensitive in 24 cases and resistance in 34 cases. In old group the results were sensitive in 24 cases and in 73 cases were resistant, the resistance to rifampicin was more in the old group (73 cases) than in new group (34 cases) there was asignificance difference with P- value = 0.024 .
The testing of DST of INH drug in new group was sensitive in 16 cases and resistance in 16 cases. The results of old group shows that 81 samples were resistant to INH .Although the resistance to INH was more in the old group (81 cases) than in new group (42 cases) there was no statistical significant difference between the two groups (P- value = 0.075).
The result of DST of streptomycin in new group was sensitive in 32 cases and showed resistance in 26 cases. In old group the results were sensitive in 43 cases and in 54 cases were resistant, although the resistance to streptomycin was more in the old group (54 cases) than in new group (26 cases) there was no statistical significant difference with
P- value =0.127

Table 5: Patterns of DST according to gender and patients groups.

DST patterns	Male	female	P	New group	Old group	P
all senstive	11(10.4)	7(14.3)	0.59	11(19.0%)	7(7.2%)	0.1
mono	17(16.0)	7(14.3)		10(17.2%)	14(14.4%)	
MDR	14(13.2)	3(6.1)		6(10.3%)	11(11.3%)	
poly	53(50.0)	29(59.2)		24(41.4%)	58(59.8%)	
Other group	11(10.4)	3(6.1)		7(12.1%)	7(7.2%)	
total	106(100)	49(100)		58(100)	97(100)	

The pattern of drug resistance according to patient group show high frequency of polyresistance 82 cases in both patient group(new and old) and low frequency in other group of resistance (14 cases).It showed that MDR type of resistance in total of 17 cases .Both groups of patients showed sensitive result in a total of 18 cases.

DISCUSSION:

This study is the first one that was conducted after starting the new referral system of samples to the Central laboratory(NRL) instead of sending patients themselves .During the study period, samples from 155 patients(58 in the new and 97 in the old group)were studied, with a mean age of (36.06±12.41) in new group and (37.20±12.88) in the old group.There was no statistically significant difference between the two groups regarding age and sex(table1 and 2).More than half of the patient are residents in Bahgdad and the other half distributed allover the Iraqi governerates(table 3).
In this study we found ethambutol resistant in 20(34.5%)of samples in the new group and in

49(50.5%) of samples in old group with statistically significant difference between the two groups(P =0.037)(table 4).These results are different from that of a large 5 years Indian study which showed ethambutol reistant in 21.7% of 673 samples [8].Also differ from that of Suadian study which showed ethambutol reistant in 22 out of 214 (10.3%) of new patients and 5 out of 108(4.6%)in samples of previously treated patients [9] .Ethambutol resistance in our study was much higher than the result of another study in Turky in which it was 2.4%(n:863) [10].

On the other hand,our study showed that resistance to rifampicin in the new group was 58.6% while in the old group was 75.3%. with statistically significant difference between the two groups(P =0.024).These figures are much higher than that of rifampicin resistance in Saudia Arabia study as it was 7/214(3.3%) in the new patients and 3/108(2.8%)[9] also higher than what is seen in Turkey 10.6%(683).However these findings are comparable to what is seen in India :74.4%[8].

In regard to the INH drug resistance it seen in 72.4% of samples in new group and in 83.5% of samples in old group.However, this difference between the two groups was not statistically significant (P= 0.075).These results are much higher than what is seen in Saudi Arabia (20.6% in new group and 22.2% of samples in old group) and Turkey(14.4%) [9,10].Also are higher to what is seen in India where resistance to INH also high(53.2%) [8].

This study showed that reistance to streptomycin present in 44.8% of new group samples and in 55.7% of old group samples with no significant difference between the two groups(p=0.127).This is different from what is seen in Saudi Arabia (32.2% in new group and 19.4% in old group samples) and Turkey (21.1%) but consistent with what is seen in India (70%) [8].

This study demonstrated that 11.6% of samples (18 of 155 sample) from patients in both groups are sensative to all drugs { 11sample (19.0%) of new group and 7(7.2%) of old group}(table 5). Also 15.5% (24/155) of the total samples were resistant to one drug{ 10(17.2%)of new group and 14(14.4%)of old group}.The study showed that 11%(17/155) of the total samples had multidrug resistance MDR{ 6(10.3%)of new group and 11(11.3%)of old group} . However,the study demonstrated that 82/155 (52.9%) of the total samples had polydrug resistance { 24(41.4%)of new group and 58(59.8%)of old group}. The trend of drug resistance in this study was consist with multiple drug resistance rather than mono drug resistance ,with shafting of drug resistance cases towards old group patients in frequency of 137 cases in contrast with new cases in frequency of 47 cases. As expected from this study the male to female ratio for drug resistance was (95/42)(2.3:1). There was no statistically significant difference between both gender and between new and old groups regarding the patteren of drug resistance(p>0.05).The results of our study are different from that found in Saudi Arabia which demonstrate higher numbers of samples that had MDR(22%) and monodrug resistance(60%) and much lower number of samples that demonstrate polydrug resistance(16%) [9].Also it differ from the result of turkish study which showed higher number of monodrug resistance (32%) and less number of MDR(6.6%) and polydrug resistance(4.4%) [10].

The result of our study demonstrate higher figures of drug resistance to antituberculosis drugs than what is estimated by the Global Project Anti-tuberculosis Drug Resistance Surveillance (GPADRS) which reported the burden of DR-TB in 138 settings in 114 countries [11,12,13].However .this difference may not necessarily reflect actual higher incidence of antituberculosis drug resistance in our country rather than that it may reflect under estmation of the proplem by that survey because these survey data, although extremely valuable, suffer from several limitations described by Cohen T et al [14].

Our study demonstrate many significant findings.It showed that 86 out of 97 (88.7%) of samples in the old group had no MDR pattern .All of these samples were wrongly used to be considered as MDR tuberculosis before application of DST in National Research Lab.(NRL) and all of these patients were mismanged .Furthermore ,7/79(7.2%) of those patients had no drug resistance to any of the 1st line drugs and 14/97(14.4%) of them had drug resistance to only one drug.

CONCLUSION:

The study showed that about 11% of patients with pulmonary tuberculosis in this sample of patients from all Iraqi governerates had multidrug resistance MDR tuberculosis and most of patients who were considered to had MDR Tuberculosis before of implication of DST in National Research Lab.(NRL) actually had no MDR Tuberculosis.

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