Original Article

Resistance of anti-tuberculosis drugs among pulmonary tuberculosis patients in Yemen

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Summary:

Background: Tuberculosis (TB) is still a major global public health problem worldwide. The global prevalence of Mycobacterium (M tuberculosis) infection has been estimated in 32% of the world population with more than 8 million new cases diagnosed each year.

Objective: To estimate drug resistance among previously treated tuberculosis patients, focusing on multi-drug resistant strains at two time intervals (2002 and 2009) in Yemen.

Materials and Methods: A total of 192 M tuberculosis complex isolates were collected from patients with positive sputum smear who had been treated previously with the four main anti- tuberculosis drugs for more than two months. The isolates were identified by their colonial morphology, pigmentation, shapes on Ziehl-Neelsen smears, growth on Löwenstein-Jenson medium and biochemical tests as niacin and nitrate tests. A proportional method was used for the in vitro drug susceptibility testing.

Results: Of the 192 M tuberculosis complex tested isolates, 55 (28.7%) were resistant to one or more drug; 20 (10.4%) were resistant to one drug, 13 (6.8%) to two drugs, 13 (6.8%) to three drugs and 9 (4.7%) to four drugs. Regarding the resistance to an individual drug, out of 192 tested isolates, 36 (18.7%) were resistant to rifampicin, 34 (17.7%) to isoniazid, 33 (17.2%) to ethambutol and 18 (9.4%) to streptomycin and these results were without a statistical significance. The incidence of multidrug resistance against rifampicin and isoniazid with or without other drugs was 13.5% among the tested M tuberculosis complex strains and this result was also without a statistical significance.

Conclusion: The results of this study revealed nearly similar drug resistance patterns for the tested isolates in comparison with previous findings of 2002 and the emergence of more multi-drug resistance M tuberculosis complex strains after a time interval in Yemen.

Key words: Tuberculosis, resistance, anti-tuberculosis drugs, MDR, Yemen.

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Introduction:

Tuberculosis (TB) is still a major global public health problem worldwide. The global prevalence of Mycobacterium tuberculosis (M tuberculosis) infection has been estimated in 32% of the world population with more than 8 million new cases diagnosed each year, 95% of them are in developing countries (1), resulting every day in more than 23000 people developing active TB and about 5000 deaths (2). In 2003, according to World Health Organization, 8.8 million new cases of TB were reported and 1.7 million deaths were attributed to the disease (3).

Yemen as any other developing country, tuberculosis is still one of the major public health problems and its socio-economic burden is obviously reflected on public health services although several efforts have been made to control this problem with the assistances of WHO and other international agencies. The re-emergence of tuberculosis over the two past decades has been accompanied by increasing drug resistant strains (4). Drug resistance in M tuberculosis occurs by mutation only, and there is no evidence of any resistance transfer factor or other genetic mechanisms operating (5, 6). Primary resistance is defined as the presence of drug resistant strains to one or more anti-tuberculosis drugs in tuberculosis patients who have never received prior treatment. Acquired resistance is defined as the presence of drug resistant to one or more drugs which occurs during the treatment, usually as a result of non adherence to the recommended regime or incorrect prescribing (7, 8). Multi-drug resistance (MDR) is defined as resistance to both Isoniazid (INH) and Rifampicin (RMP) with or without resistance to additional agents. INH and RMP represent the most potent drug combination against M tuberculosis (6). Therefore, MDR tuberculosis represents a formidable challenge to TB control in several settings (5).

In Yemen there is little reliable data about anti-tuberculosis drug resistant patterns, therefore, the present study was conducted in order to estimate drug resistance among previously treated tuberculosis patients, focusing on MDR strains at two time intervals.

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Materials and Methods:

A total of 192 clinical isolates of M tuberculosis complex collected from previously treated patients were enrolled in this study during the year 2009. The patient's age ranged from 14-70 years old.

M tuberculosis strains from patients with positive sputum smears who had been treated with Isoniazid (INH), Rifampicin (RMP), Streptomycin (SM) and Ethambutol (ETM) were isolated on Löwenstein-Jensen medium (L-J). M tuberculosis strains were identified by their typical colonial morphology, pigmentations, shapes on Ziehl-Neelsen smears, growth on PNA–L-J medium and biochemical reactions including niacin, nitrate tests according to Collee et al (7).

A proportion method described by WHO (9) was used for in-vitro drug sensitivity testing against INH, RMP, SM and ETM. All drugs were dissolved in distilled water, except rifampicin which was dissolved in dimethyl–formide. The solutions were added to the L-J medium to give the required final concentrations. About 0.2 ml of a diluted bacterial suspension was inoculated on L-J medium with drug and without drug as control. All inoculated tubes were incubated at 37ºC for up to 4 weeks. The ratio between the number of colonies in the tube containing the drug and the colonies in the tube without drug showed, whether the strain was susceptible or resistant to the tested drugs (> 1 % usually resistant).

The data were analyzed using Statistical Package of Social Science Program (SPSS, version 10). P-values < 0.05 were considered statistically significant.

Results:

This study showed that out of the total 192 studied strains of M tuberculosis, 55 (28.7%) were resistant to one or more drug, whereas the study that was performed in 2002 revealed 35 (35%) strains of M tuberculosis (10).

Table 1: Distribution of resistant M tuberculosis strains according to the number of drugs at two time intervals, 2002 and 2009.

Drugs	2002 Studied strains (100)		2009 Studied strains (192)		χ^2	p
	No.	# %	No.	#%		
One drug	14	14	20	10.4	0.82	0.36
Two drugs	12	12	13	6.8	2.4	0.1
Three drugs	6	6	13	6.8	0.06	0.8
Four drugs	3	3	9	4.7	0.48	0.44

#% is calculated from the total (100 and 192).

The distribution of resistant strains of M tuberculosis according to the number of drugs is presented in table 1. In 2002 (10), out of the 100 strains, 14 (14%) were resistant to one drug, 12 (12%) to two drugs, 6 (6%) to three drugs and finally 3 (3%) to four drugs. Whereas in 2009, 20 (10.4%) were resistant to one drug, 13 (6.8%) to two and three drugs and 9 (4.7%) to four drugs. This result was not statistically significant (χ^2=0.94 and p=0.42).

Table 2: Distribution of M tuberculosis strains according to their resistant to individual drugs at two time intervals, 2002 and 2009

Drugs	2002 Studied strains (100)		2009 Studied strains (192)		χ^2	p
	No.	# %	No.	#%		
Rifampicin	20	20	36	18.7	0.07	0.8
Isoniazid	23	23	34	17.7	1.2	0.2
Streptomycin	27	27	33	17.2	3.9	0.04
Ethambutol	5	5	18	9.4	1.73	0.18

#% is calculated from the total (100 and 192).

Table 2 shows the distribution of M tuberculosis strains according to their resistant to individual drugs at two time intervals, 2002 and 2009,whereas in 2002, 20 (20%) were resistant to rifampicin, 23 (23%) to isoniazid, 27 (27%) to streptomycin and finally 5 (5%) were resistant to ethambutol (10). While in 2009, of the 192 tested strains, 36 (18.7%) were resistant to rifampicin, 34 (17.7%) to isoniazid, 33 (17.2%) to ethambutol and 18 (9.4%) to streptomycin. This result was also not statistically significant (χ^2=2.12 and p=0.5).

Table 3: Incidence of multi-drug resistance M tuberculosis strains at two time intervals, 2002 and 2009

Drugs	2002 Studied strains (100)		2009 Studied strains (192)		χ^2	p
	No.	# %	No.	#%		
Rifampicin + Isoniazid	9	9	26	13.5	3.2	0.05

% is calculated from the total (100 and 192).

Incidence of MDRM tuberculosis strains isolated from previously treated patients is presented in table 3. In 2009, 26 (13.5%) out of the 192 tested strains were resistant to rifampicin and isoniazid with or without other drugs. In 2002, MDR was found to be 9 (9%) (10). This result was also not statistically significant (χ^2=3.2 and p=0.05).

Discussion:

Drug resistant tuberculosis is becoming increasingly important, and it has been one of the problems in the treatment. This study was conducted in order to identify resistance to anti-tuberculosis drug and focusing on more MDR M tuberculosis strains in Yemen. The present study showed that out of 192

studied strains of M tuberculosis isolated from previously treated patients, 55 (28.7%) were resistant to one or more of the tested drugs (RMP, INH, ETM and SM). In a previous study in 2002, Al-Madhagi (10) stated that the overall incidence of drug resistance among strains isolated from previously treated patients was 35%, this slight difference in the resistant rate may be attributed to different parameters of which the sample size may play an important role and the resistance to individual drugs themselves. Several results dealing with the same problem were reported by WHO (8), some of these results showed higher resistant rates as reported in Cuba (91.3%), Latvia (73.3%), Portugal (37.6%) and Spain (29.9%). On the other hand, lower resistant rates were found in France (21.6%) (8). A study performed in Saudi Arabia showed a rate of 15% (11). Drug resistant rates and their variations in different countries might be attributed to different regimens of treatment used and health educational levels. As regards the individual drug resistance, in this study the incidence of resistant to RIM, INH, STM and ETM were 18.7%, 17.7%, 17.2 and 9.4% respectively. It is noted that the highest resistant rate was found with RIM and the lowest with ETM. The resistance of STM (17.2%) in this study appears to be less than that of a previous study (27%) (10), this may be due to that streptomycin is less used with treatment of tuberculosis in recent years. In contrast, Al-Hajjaj et al (12) in a study carried out in Saudi Arabia in 2001 stated that the incidence of resistance to a drug was more common with STM (27.4%) and less common with ETM (1.8%). In another country neighboring Yemen, Ethiopia, the incidence of resistance to a drug was most common with INH (46%), and least common with ETM (5%) (13). In Egypt, the highest resistant pattern was found with INH (69.2%) and the lowest with INH (15.4%) (14). In a study conducted in Iraq, 2009, out of 53 patients with positive pulmonary smears, of whom 38 (71.7%) were new TB cases and 15 (28.3%) were previously treated patients. Four of the 38 new cases (10.5%) had drug resistant strains, of which 3 (7.9%) were MDR. Eight (53.3%) of the 15 previously treated patients had resistant strains, of which 7 (46.7%) were MDR. Overall, 10 patients (18.7%) were MDR-TB (15). MDR rates were found in France (0.5%), England and Wales (0.6%) and in south east England, MDR rate was 8.9% (16). A study conducted by Jain et al in India found that the prevalence of MDR was 19.8 %, initial and acquired were found to be 13.2 and 25.5 % respectively (17). MDR rates were found in South East Asia 14.9% (18) and in Uganda 12.7% (19). These results nearly agreed with those in this study. Due to the presence of a remarkable drug resistance among the tested isolates and the emergence of more MDR strains in Yemen, a better regimen for treatment should be introduced on a wider scale such as that recommended by WHO and known as DOTS strategy (20). This strategy provides the tuberculosis patient with all the necessary requirements for cure and places this patient at the center of tuberculosis control activities. In addition, the use of anti-tuberculosis drugs should be restricted to tuberculosis patients only.

Authors Contributions:

Anwar Kassem Al-Madhagi: study conception, design.

Khaled Abdul Karim Al-Moyed: acquisition of data analysis, interpretation of data.

Ahmed Mohamed Al-Haddad: drafting of manuscript, critical revision.

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