Mycobacterial Diseases
ISSN: 2161-1068

Albarouni AS et al., J Mycobac Dis 2014, 4:3
DOI: 10.4172/2161-1068.1000154

Research Article Open Access

Drug Susceptibility Patterns of *Mycobacterium tuberculosis* Isolated from Patients with Pulmonary Tuberculosis in Tripoli-Libya

Abdulmonam S Albarouni[1,2], Naima B Aboguttaia[1], Wedad M Alamri[1], Alkhnsaa M Fungi[1], Mohammed Furjani[1] and Abdulaziz Zorgani[1,3*]

[1]*TB-Laboratory, National Centre for Diseases Control, Tripoli-Libya*

[2]*Faculty of Medical Technology, Tripoli-Libya*

[3]*Department of Microbiology and Immunology, Faculty of Medicine, Tripoli University, Tripoli-Libya*

[*]**Corresponding author**: Abdulaziz Zorgani, Tripoli University, Tripoli-Libya, Tel: 00218913718561; E-mail: zorgania@yahoo.com

Rec date: Mar 22, 2014; **Acc date:** Apr 28, 2014; **Pub date:** May 02, 2014

Abstract

Background: The emergence and spread of drug resistant and multidrug resistance are a global health problem. The susceptibility patterns of *M. tuberculosis* isolates against anti-tuberculosis drugs forms an important aspect of the control programs at the local level. The aim of this study was to determine the pattern of the susceptibility of drugs to *M. tuberculosis* isolates from patients with pulmonary tuberculosis in Tripoli, Libya.

Methods: Drug Susceptibility Test (DST) was performed on 261 isolates of *M. tuberculosis* by BD BACTEC MGIT 960 SIRE system. The drugs tested were: isoniazid (INH), rifampicin (RIF), streptomycin (SM) and ethambutol (EMB).

Results: All isolates (261) were confirmed as *M. tuberculosis* complex and showed different resistance patterns: 8.8% to INH; 5.7% to RIF; 8.8% to SM; and 9.0% to EMB. Rifampicin was the lowest detected resistance first-line antibiotics studied. One drug resistant was observed in 18.0%; 3.8% were resistant to two drugs; and 2.3% were resistant to a combination of three-drugs. Of the total 261 cases, 217 were designated as new untreated patients and 44 as previously treated patients. In terms of resistance to any drug, there was a significant difference between the two categories (P<0.014). However, there was no significant difference between new and previously treated patients in relation to one drug resistant (P=0.4). Meanwhile, there was a significant difference in relation to two drug resistant (P<0.005), Nine (20.5%) of the isolates designated multi-drug resistant (MDR) were obtained only from previously treated patients. None of newly treated cases had isolates resistant to three-drugs nor MDRs.

Conclusion: This preliminary study indicated the low prevalence of drug resistance *M. tuberculosis* (MTB) among previously treated patients in Tripoli.

Keywords: *Mycobacterium tuberculosis*; Drug susceptibility test; Drug resistance; Multidrug resistance; Libya

Abbreviations:

TB: Tuberculosis; MDR: Multi-Drug Resistant; MTB: *M. tuberculosis*; DST: Drug Susceptibility Test; INH: Isoniazid; RIF: Rifampicin; SM: Streptomycin; EMB: Ethambutal; WHO: World Health Organization; NCDC: National Center for Disease Control

Introduction

Tuberculosis (TB) is a major cause of morbidity and mortality worldwide, especially in the developing countries. World health organization (WHO) estimates that 8.8 million new cases and 1.1 million deaths from TB occurred in the year 2010. Most of the estimated numbers of cases were in Asia (59%) and Africa (26%) [1]. In Libya, the prevalence of TB cases is estimated in the period of 2010 at 40 cases per 100.000 populations and the incidence of 53 cases per 100.000 populations [1]. Globally, there were an estimated 630,000 cases of multi drug resistant (MDR) TB (range, 460 000–790 000) among the world's 12 million prevalent cases of TB in 2011. Almost 60% of MDR-TB cases worldwide are estimated to occur in India, China, the Russian Federation and South Africa [2].

MDR-TB is caused by strains of *M. tuberculosis* (MTB) that are resistant to at least isoniazid and rifampicin, the most effective first-line TB drugs. The use of drugs in suitable doses and duration can control the disease to a great extent. However, inadequate treatment, due to poor patient compliance, inappropriate therapy regimen, and irregular drug supplies are important factors in development of drug resistance [3].

A national surveillance and analysis of local rates of drug resistance patterns in MTB are an essential tool for monitoring of the effectiveness of TB control programs and prevention of the dissemination of resistant strains in the community [4]. Although, drug resistance has been reported from different countries, according to our knowledge there is no information to evaluate the current status of MDR-TB in Libya. In this context, the aim of this study was to provide a preliminary assessment rate and patterns of MTB resistance of isolates obtained from patients diagnosed with pulmonary tuberculosis.

Material and Methods

This study was conducted from February 2010 to January 2011 in the TB reference laboratory at the National Center for Disease Control (NCDC), Tripoli, Libya. During this period, 3,590 sputum specimens were investigated in 1,029 patients clinically suspected of suffering from pulmonary TB.

All sputum specimens were routinely examined by Ziehl-Neelsen staining and culture analysis tests. Each sputum specimen was digested and decontaminated by the N-acetyl-L-cystine-NaOH (Mycob prep-Becton Dickinson) method according to the standard procedure. After decontamination, 500 µl of the processed specimen was inoculated into an MGIT culture tube containing both 10% OADC (oleic acid, albumin, dextrose, and catalase; Becton Dickinson) and 0.8 ml of PANTA antimicrobial supplement (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin; Becton Dickinson). After that, inoculated MGIT tubes were incubated in the MGIT culture system until growth was seen or up to 42 days of no growth. The isolates were identified as MTB by BD MGIT TB identification test and characterized by certain biochemical tests such as niacin accumulation and susceptibility to para-nitrobenzoic acid (PNB) [5].

Drug susceptibility testing was performed on MTB isolates using the BACTEC MGIT 960 liquid culture system in accordance with the manufacturer's recommendations. For each isolated, 5 tubes were prepared: four of the tubes contained the anti-tuberculosis drugs (BACTEC MGIT SIRE; Becton Dickinson), and one was a drug-free growth control. To all tubes, 0.8ml of growth supplement (BACTEC MGIT 960 SIRE supplement; Becton Dickinson) was added and 100µl of drug stock solution was added to each drug containing tube. The final critical concentrations of each anti-tuberculosis in the test tubes was 0.1µg/ml INH, 1.0µg/ml RIF, 1.0µg/ml SM and 5.0µg/ml EMB. All of the drugs containing tubes were then inoculated with 0.5ml of the positive broth culture. The drug-free control was inoculated with 0.5ml of a 1:100 dilution of the positive culture broth in sterile distilled water. Drug susceptibility results were interpreted on the same day that distinct growth was seen in the control media. A P value <0.05 was considered as significant. Open Epi software (Epi Info™ 7,) was used for all statistical analyses.

Results

Of the 3,590 sputum specimens collected from clinically suspected TB patients, 599 (16.7%) were proven cultures positive for MTB. A drug susceptibility test was performed only on 261 MTB isolates randomly selected due to economic measures. There were 218 (83.5%) males with the mean age of 35 years and 43 (16.5%) were females with the mean age of 40 years, with a male: female ratio of 5:1. The majority of cases 223 (85.4%) were from Libyan patients, and 38 (14.6%) were African and Asian patients.

Out of 261 isolates, 198 (75.9%) were sensitive to all four drugs, whereas 63 (24.1%) were resistant to one or more of the drugs. The isolates showed different resistance patterns to four drugs tested as following: 23 (8.8%) to INH; 15 (5.7 %) to RIF; 23 (8.8%) to SM; and 25 (9.0%) to EMB, rifampicin was the lowest detected resistant to the studied first-line antibiotics. Forty seven (18.0%) of isolates were resistant to one drug, 10 (3.8%) were resistant to two drugs and 6 (2.3%) were resistant to a combination of three-drugs. One drug resistance was observed in all four drugs tested as following: RIF was the lowest (1.5%) exceeded only by INH (3.8%), SM (5.4%), and EMB 18.0%) (Table 1). Resistance to RIF was found associated with two drug resistance and three-drug resistance, INH was involved in all patterns of three-drug resistance combinations.

The comparison of the resistance patterns of new untreated cases (217) and previously treated cases (44) are shown in Table 2. There was a significant difference between the two categories (P<0.014) in terms of resistance to any drug. However, there was no significant difference between new and previously treated patients in relation to one drug resistant (P=0.4), the majority were resistant to EMB, SM and INH. Meanwhile, there was significant different in relation to two drug resistant (P<0.005) nor MDR. None of newly treated cases had isolates resistant to three-drugs. Nine (20.5%) of the MDR isolates were found only in previously treated patients.

Resistance pattern	No. of resistant isolates (%)
Resistance to any drug	63 (24.1)
One drug resistance	
INH	10 (3.8)
RIF	4 (1.5)
SM	14 (5.4)
EMB	19 (7.3)
total	47 (18.0)
Two drug resistance	
INH+RIF	4 (1.5)
INH+SM	1 (0.4)
INH+ EMB	2 (0.8)
SM+EMB	2 (0.8)
RIF+SM	1 (0.4)
total	10 (3.8)
Three drug resistance	
INH+RIF+EMB	1 (0.4)
INH+RIF+ SM	4 (1.5)
INH+SM+EMB	1 (0.4)
total	6 (2.3)
MDR*	9 (3.4)

Table 1: Drug resistant patterns to four anti-tuberculosis drugs. *MDR: Multi-drug resistant: resistance to both INH and RIF with or without resistance to other drugs

Resistance pattern	No. of new untreated patients (%)	No. of previously treated patient (%)	P value
resistance to one drug or more	46 (21.2)	17 (38.6)	<0.014
One drug resistance	41 (18.9)	6 (13.6)	0.4
Two drug resistance	5 (2.3)	5 (11.4)	<0.005

Three drug resistance	0	6 (13.6)	<0.0000001
Multidrug-resistance	0	9 (20.5)	<0.0000001

Table 2: Comparison of drug resistant pattern among new untreated patients and previously treated patients

Discussion

The emergence of MDR-TB poses an important challenge for tuberculosis treatment and control programs [6]. Strengthening the surveillance and analysis of local rates of TB drug resistance can be helpful in the detection and monitoring the effectiveness of tuberculosis programs worldwide. In Libya, like many developing countries, no national study has been conducted to evaluate the current status of MDR-TB. Therefore, this study was undertaken to determine the resistance pattern of MTB isolates among patients with pulmonary tuberculosis in Tripoli.

In this study, 24.1% of all isolates of MTB were found resistant to one or more of the first-line drugs, of these 46 (21.2%) isolates were detected among new untreated patients, and 38.6% isolates among previously treated patients. Overall, the tested isolates showed different resistance patterns to four drugs as following: 8.8% to INH; 5.7% to RIF; 8.8% to SM; and 9.0% to EMB, rifampicin was the lowest detected resistant to the studied first-line antibiotics. These results differ from studies carried out in other developing countries. In Pakistan, drug resistance to any drug was high 71.9%. Meanwhile, resistance to first-line drugs was reported to INH, RMP, SM, EMB were 51.2%, 15.4%, 3.9%, and 13.3% respectively [7]. Yeboah-Manu et al. [8] from Ghana showed that the pattern of drug resistance was detected in 38.8% of the tested isolates and resistance to INH was 14.9%, to RIF was 6.6%, to SM was 31.4% and to EMB was 3.3% [8]. Similarly, Ndungu et al. [9] reported that drug resistance to all drugs tested were 30.1% in Kenya, and resistance to INH, RMP, SM and EMB were 30.2%, 1.4%, 11.6% and 4.5 % respectively [9]. All these studies may reflect the variation in the studied population and the commitment of regional TB program.

The early detection of resistance to first-line anti-TB agents is essential for the efficient treatment and control of MDR-TB strains. In this study, resistance of MTB to one drug was detected in 18.0% cases, 15.7% of which were from new untreated patients. The highest rate of one drug resistance was observed in EMB (7.3%), followed by (5.4%) to SM, (3.8%) to INH, and (1.5%) to RIF of all strains. Mono-resistance to streptomycin (14.9%) was found to be the highest proportion among first-line anti-TB drugs RIF and INH, which is consistent with WHO global surveillance report [10] as well as in other studies conducted in Denmark, Turkey, India and Ethiopia [11-14]. In addition, 3.8% and 2.3% cases had resistance to two and three-drugs respectively. RIF was found the lowest detected resistance and associated with two drug resistance and three-drug resistance. In this study, as is the case worldwide, the vast majority of MTB RIF-resistant strains are also INH-resistant.

The comparison of the resistance patterns in new untreated cases and previously treated cases has been studied worldwide [15-17]. Of the total 261 cases, 217 were obtained from new untreated patients, and 44 were previously treated patients. There was a significant difference between the two categories (P<0.014) in terms of resistance to any drug. Interestingly, there was no significant different between new and previously treated patients in relation to one drug resistant (P=0.4). On the other hand, there was significant different in relation to two drug resistant (P=0.005). None of newly untreated cases had isolates resistant to three-drugs nor MDR. Resistance against two and three-drugs was significantly higher in previous treated patients than in new untreated patients. This mode of resistance may be difficult to treat and often result in treatment failure [18].

The weighted rates of resistance in the world population are estimated among new cases at 17% for any resistance and 2.9% for MDR-TB, and among previously treated cases at 35% for any resistance and 15.3% for MDR-TB [19]. Our results remain well below the rates reported in Thailand, Vietnam and China [20-22].

MDR involving INH and RIF was found low, only in 9 (3.4%) isolates belonged to previously treated patients (acquired drug resistance). Two of the cases were foreign-borne patients (Sudanese), where the incidence of TB is high [1]. In addition, the majority 5/9 of the isolates were designated three-drug resistant. The relationship between history of receiving anti-TB treatment and MDR has been clearly described in several studies [3,9,23,24]. Surprisingly, our study noted that MDR-TB prevalence is significantly higher in male patients (6/9) which are consistent with a recent report by Abate et al. [24]. A recent report by the WHO revealed that drug resistance was present globally, and that MDR-TB ranged from 0% to 28.3% in new untreated cases and 0% to 61% in previously treated cases. 15 of the 27 countries with the highest burden of MDR tuberculosis are in WHO European region [2,10]. According to WHO report (2010), only 8/21 of the eastern Mediterranean region countries report continuously first-line anti-TB drug resistance [2]. In Libya, compulsory screening measures of all foreign-borne workers focused on the identification of active tuberculosis cases is strongly implemented. Several countries with a low incidence of tuberculosis use pre-entry screening of immigrants to detect the disease [25-28]. In our retrospective study, the following limitations are noted: no data was available from previously treated patients (failed or relapsed after the standard regimen); the method used for DST was different from the ones used in other studies; and the HIV status of the patients was unknown. Furthermore, although NCDC is the major referral TB centre in Libya, the patient sampling is small and does not represent the whole country.

In conclusion, although the findings of a low burden of drug resistance to first-line TB drugs and low MDR as compared to other parts of the world is encouraging. Nevertheless, further studies are required including different geographic locations of the country that should be receiving support from the national TB control program. It would appear that monitoring of drug resistance should be enhanced by periodic surveys to assess trends of antibiotic-resistant patterns, and to prevent transmission of MDR-TB and progression of active disease.

References

1. World Health Organization: Global tuberculosis Control. WHO/HTM/TB. 2011; Geneva.
2. World Health Organization: Global tuberculosis report; WHO/HTM/TB/2012.6, ISBN 978 92 4 1564502.
3. Bilgin S, Unsal M, Cebi HH, Akgunes A (2010) Resistance for anti-tuberculosis drugs in central Black Sea region of Turkey. Pol J Microbiol 59: 125-128.
4. Menon S, Dharmshale S, Chande C, Gohil A, Lilani S, et al. (2012) Drug resistance profiles of Mycobacterium tuberculosis isolates to first line anti-tuberculous drugs: A five years study. Lung India 29: 227-231.
5. Barouni AS, Alnajh ZB, Aboguttaia NB, Alamri WM (2012) Evaluation of the BD TBc identification test for rapid identification of

Myobacterium tuberculosis complex from positive BACTEC MGIT 960 cultures in a routine laboratory work. African J Microbiol Res. 6: 1065-1068.

6. Nuermberger EL, Spigelman MK, Yew WW (2010) Current development and future prospects in chemotherapy of tuberculosis. Respirology 15: 764-778.
7. Khoharo HK, Shaikh IA (2011) Drug resistance patterns in pulmonary tuberculosis. J Pak Med Assoc 61: 229-232.
8. Yeboah-Manu D, Asante-Poku A, Ampah KA, Kpeli G, Danso E, et al. (2010) Drug susceptibility pattern of Mycobacterium Tuberculosis isolates from Ghana; correlation with clinical response. Mycobac Dis. 2: 100-107.
9. Ndung'u PW, Kariuki S, Ng'ang'a Z, Revathi G (2012) Resistance patterns of Mycobacterium tuberculosis isolates from pulmonary tuberculosis patients in Nairobi. J Infect Dev Ctries 6: 33-39.
10. Prasad R (2010) Multidrug and extensively drug-resistant TB (M/XDR-TB): problems and solutions. Indian J Tuberc 57: 180-191.
11. Thomsen VO, Bauer J, Lillebaek T, Glismann S (2000) Results from 8 yrs of susceptibility testing of clinical Mycobacterium tuberculosis isolates in Denmark. Eur Respir J 16: 203-208.
12. Senol G, Komurcuoglu B, Komurcuoglu A (2005) Drug resistance of Mycobacterium tuberculosis in Western Turkey: a retrospective study from 1100-bed teaching hospital. J Infect 50: 306-311.
13. Kart L, Altin R, Tor M, Gulmez I, Oymak SF, et al. (2002) Antituberculosis drug resistance patterns in two regions of Turkey: a retrospective analysis. Ann Clin Microbiol Antimicrob 1: 6.
14. Gupta PR, Singhal B, Sharma TN, Gupta RB (1993) Prevalence of initial drug resistance in tuberculosis patients attending a chest hospital. Indian J Med Res 97: 102-103.
15. Lukoye D, Adatu F, Musisi K, Kasule GW, Were W, et al. (2013) Anti-tuberculosis drug resistance among new and previously treated sputum smear-positive tuberculosis patients in Uganda: results of the first national survey. PLoS One 8: e70763.
16. Skrahina A, Hurevich H, Zalutskaya A, Sahalchyk E, Astrauko A, et al. (2013) Multidrug-resistant tuberculosis in Belarus: the size of the problem and associated risk factors. Bull World Health Organ 91: 36-45.
17. Sindani I, Fitzpatrick C, Falzon D, Suleiman B, Arube P, et al. (2013) Multidrug-resistant tuberculosis, Somalia, 2010-2011. Emerg Infect Dis 19: 478-480.
18. World Health Organization. Guidelines for the management of drug-resistant tuberculosis. WHO 1997.
19. WHO/IUATLD, WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance: Anti-tuberculosis drug resistance in the world, Fourth global report. Geneva; 2008.
20. Riantawan P, Punnotok J, Chaisuksuwan R, Pransujarit V (1998) Resistance of Mycobacterium tuberculosis to antituberculosis drugs in the Central Region of Thailand, 1996. Int J Tuberc Lung Dis 2: 616-620.
21. Huong NT, Lan NT, Cobelens FG, Duong BD, Co NV, et al. (2006) Antituberculosis drug resistance in the south of Vietnam: prevalence and trends. J Infect Dis 194: 1226-1232.
22. Yang Y, Li X, Zhou F, Jin Q, Gao L (2011) Prevalence of drug-resistant tuberculosis in mainland China: systematic review and meta-analysis. PLoS One 6: e20343.
23. Yu CC, Chang CY, Liu CE, Shih LF, Hsiao JH, et al. (2010) Drug resistance pattern of mycobacterium tuberculosis complex at a medical center in central Taiwan, 2003-2007. J Microbiol Immunol Infect 43: 285-290.
24. Abate D1, Taye B, Abseno M, Biadgilign S (2012) Epidemiology of anti-tuberculosis drug resistance patterns and trends in tuberculosis referral hospital in Addis Ababa, Ethiopia. BMC Res Notes 5: 462.
25. Lords Hansard statement. Immigration: pre-entry screening for tuberculosis. 2010.
26. Lowenthal P, Westenhouse J, Moore M, Posey DL, Watt JP, et al. (2011) Reduced importation of tuberculosis after the implementation of an enhanced pre-immigration screening protocol. Int J Tuberc Lung Dis 15: 761-766.
27. Department of Immigration and Citizenship, Health Policy Section. Instructions for medical and radiological examination of Australia visa applicants. Canberra, ACT, Australia: Department of Immigration and Citizenship, 2009.
28. Minister of Public Works and Government Services, Health Management Branch. Handbook for designated medical practitioners. Ottawa, ON, Canada: Minister of Public Works and Government Services, 2009.