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Patterns of Anti-tuberculosis Drug Resistance among Pulmonary Tuberculosis Patients in Diyala- Iraqi

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ABSTRACT

Multidrug-resistant tuberculosis (MDR-TB), is a growing clinical and public-health concern. World Health Organization (WHO) has recognized MDR and Extended drug resistant tuberculosis (XDR-TB) as a major challenge to be addressed as part of the stop TB strategy, launched in 2006.This cross sectional study was conducted in the Consultation Clinics for Chest and Respiratory Diseases in Diyala province for the period from the 1st of September/2011 to the 31st of July/2012, to identify the patterns of anti-tuberculosis drug resistance among pulmonary tuberculosis (PTB) patients in Diyala province and its relevance with some sociodemographic factors of PTB patients.A total of 150 patients were included in this studychosen by random selection. Sputum and bronco-alveolar washing samples were collected and submitted to direct Zeil-Neelson stain. Thereafter, each specimen was cultured on Lowenstein-Jenson and Stonebrick media in duplicate. Identification of *M.tuberculosis* was based on standard bacteriological and biochemical criteria. Drug susceptibility testing of *M. tuberculosis* isolates against the first and second lines of antituberculous drugs using the conventional agar proportional method according to the manufacturer's instructions (Himedia, India). Collected data were statistically analyzed and *P* value was considered significant if it is < 0.05.Fifty (33.3%) isolates of *M. tuberculosis* were recovered by culture. Of these, 6 (12%) isolates has monoresistance and 20 (40%) isolates were multi-drug resistant to the first line drugs. The overall rate of MDR-TB was 13.3% of the PTB patients, and 10.7% of these were among previously treated PTB cases. Susceptibility tests to the second line antituberculous drugs revealed that 2 of the MDR isolates were pre-XDR, 4 isolates were polyresistant, and 2 isolates were monoresistant. Most of the resistant isolates were a recovered from patient with relapsed PTB.The XDR-TB is still rare in Diyala province. The rate of MDR-TB is within the range recognized by the WHO.

Keywords: Pulmonary tuberculosis, Multi-drug resistanttuberculosis, Extended drug resistant tuberculosis.

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Abbreviations: PTB- pulmonary tuberculosis, MDR-TB- multidrug resistant tuberculosis, XDR-TB- Extended drug resistant tuberculosis.

1. INTRODUCTION

Despite an important progress towards global targets to halt and reverse the tuberculosis (TB) epidemic by 2015, however, the global burden of TB remains enormous. In 2011, there were an estimated 8.7 million new cases of TB and 1.4 million people died, 95% of them occur in low- and middle-income countries. Worldwide, 3.7 % of new cases and 20% of previously treated cases were estimated to have multi –drug resistance (MDR-TB) (WHO, 2012). MDR-TB is a form of TBcausedby bacteria that do not respond to, at least, isoniazid and rifampicin, the two most powerful, first-line (or standard) anti-TB drugs (Chiang et al., 2010). MDR-TB results from either infection with organisms which are already drug-resistant or may develop in the course of a patient's treatment. The primarycauses of MDR-TB are inappropriate treatment, inappropriate or incorrect use of anti-TB drugs, or use of poor quality medicines (Jainand Dixit, 2008). The highest proportions of TB patients with MDR-TB are in Eastern Europe and Central Asia (Royce, 2013) .On the other hand, XDR- TB is a form of TB caused by organisms that are resistant to isoniazid and rifampicin (i.e. MDR-TB) as well as any fluoroquinolone and any of the second–line anti-TB injectable drugs (Amikacin, Kanamycin or Capreomycin). These forms of TB do not respond to the standard six month treatment with first-line anti-TB drugs and can take two years or more to treat with drugs that are less potent, more toxic and much more expensive (WHO, 2006). XDR-TB has been reported by 84 countries, the average proportion of MDR-TB cases with XDR-TB is 9% (WHO, 2012). The WHO has recognized MDR/XDR-TB as a major challenge to be addressed as part of the Stop TB strategy, launched in 2006 (WHO, 2006).

2. PATIENTS AND METHODS

The present cross-sectional study was conducted in the Chest and Respiratory Diseases Clinic (CRDC) in Baquba-Diyala province for the period from September/2011 to August/2012. A total of 150 patients were included. All patients were either referred to the CRDC for the first time on the basis of inclusion criteria (cases clinically suspected as having PTB), or those previously treated as PTB patients for different intervals.Patients were categorized into newly diagnosed, follow-up, relapse, chronic and contacts according to the WHO criteria (WHO, 1997). Ethically, the human privacy was respected; so, that a written informed consent was obtained from each patient prior to collection of sample. Special questionnaire was pre-constructed for this purpose including information of age, sex, residence, type of specimen, other information concerning disease history and duration of treatment were subtracted from records. Sputum and bronco-alveolar washing samples were collected in wide opening disposable containers with tight cover.

All samples were processed using NALC-NaOH method (Kent and Kubica, 1985). Specimens were submitted to direct Zeil-Neelson stain (Fuji Ki, 1998). Thereafter, each specimen was cultured on slants of Lowenstein-Jenson and slants of Stonebrick media in duplicate (Grangeand Mycobacterium, 2007). Cultures were incubated in 37^0C for 6 weeks. Identification of *M. tuberculosis* was based on colonial morphology, ZN stain, and biochemical tests (Catalase, Niacin production, and Nitrate reduction) and p-nitrobenzoic acid (PNB) tests (Thomas, 1998). The isolates were tested against the first and second lines of antituberculous drugs using the proportional method according to the manufacturer's instructions (Himedia, India). The first line antituberculous drugs include; Isoniazid 0.2 mcg/ml, Ethambutol 2 mcg/ml, Pyrazinamide 200 mcg/ml, Rifampicin 40 mcg/ml, and Streptomycin 4 mcg/ml. While the second line drugs include; Kanamycin 30 mcg/ml, Amikacin 700 mcg/ml, Ethionamide 20 mcg/ml, D-Cycloserine 30 mcg/ml, Clarithromycin 8 mcg/ml, Ciprofloxacin 12.5 mcg/ml, P-amino salicylic acid 2.5 mcg/ml, and Rifabutin 0.5 mcg/ml. MDR-TB was defined as tuberculosis disease caused by a strain of *M. tuberculosis* that was resistant to at least Rifampicin and Isoniazide. Pre-XDR was defined as disease caused by a strain resistant to Rifampicin and Isoniazide and either a fluoroquinolone or a second-line injectable drug, but not both. XDR-TB was defined as TB with resistance to at least Rifampicin, Isoniazid, a fluoroquinolone and one of three second-line injectable drugs; Kanamycin, Amikacin, and Clarithromycin [5]. The resistant rate was calculated by dividing the No. of colonies on drug media on the No. of colonies on control media multiply by 100, and the isolates were considered resistant when the results were > 1%. Statistical analysis was done using the Statistical Analyses System version 7, and P value was considered significant if it is < 0.05.

3. RESULTS

The results of culture in relation to the demographic characteristics of the studied PTB cases were shown in table 1. Males constituted 60% of the cases, and those who were culture positive foundin (35.5%) while (64.4%) were culture negative. Females constitutes(40%) of the cases, (30%)of themwereculture positive.Males were found to be harbor

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Table 1

Results of culture and MDR isolate in relation todemographic characteristics of the study case

Demographic	Culture (+)ve	Culture (-)ve	MDR* isolate
Variables	**No. (%)**	**No. (%)**	**No. (%)**
Gender **Male**	32 (35.5)	58 (64.4)	11 (55)
Female	18 (30.0)	42 (70.0)	9 (45)
Age / year **<20**	2 (4.0)	12(12.0)	0 (0.0)
21- 40	27 (54.0)	29 (29.9)	12 (60)
41-60	15 (30.0)	34 (34)	6 (30)
> 60	6 (12.0)	25 (25.0)	2 (10.0)
Total	50 (100)	100 (100)	20 (100)

*MDR =Multi drug resistance.

Table 2

Results of culture and MDR according to patient's categories

Patients categories	Culture (+ve) No. (%)	Culture (-ve) No. (%)	MDR isolate No. (%)	ATB drugs & type resistance
Newly	18 (36.0)	75 (72.0)	4 (20.0)	KM,CLR ETA,CS,PAS (Pre-XDR*)
Follow up	0 (0.0)	5 (5.0)	0 (0.0)	
Relapse	18 (36.0)	2 (2.0)	14 (70.0)	KM,RFB(Pre-XDR*) , ETA,CS, CLR, RFB(poly***) REF (Mono**) CS (Mono**) ETA,CS,RFB (poly***)
Chronic	12 (24.0)	20 (20.0)	2 (10.0)	ETA, CS, CLR, RFB(poly*)
Contacts	2 (4.0)	1 (1.0)	0 (0.0)	
Total	50 (100)	100 (100)	20 (100)	

*Pre-XDR **Mono-resistance ***Poly-resistance

Table 3

Results of the biochemical tests of the M.TB. Isolates

Result	Pyruvate	TCH	PNB	Niacin	Nitrate	Catalase	
						25 ^{0}C	68 ^{0}C
Positive	2	49	1	48	49	50	0
Negative	48	1	49	2	1	0	50

(55%) of the MDR isolates, while females' harbor(45%) of these isolates.The distribution of culture results according to the age of patients,the highest culture positive (54%) were among those 21-40 years old, and the same age group harbor (60%) of MDRisolate.

Table 2 shows the results of positive culture according to patient's categories. A total of 150 patients with PTB were included. Of them 98 (65.3%) had primary PTB, and 52 (34.7%) were previously treated PTB. They are as follows; 90 were newly diagnosed, 5 werefollow-up, 20 wererelapse, and 32 chronic cases, beside a 3 contacts of PTB patients. 43 (28.7%) were sputum smear (SS) positive and 50 (33.3%) were positive by culture. Concerning MDR isolate the highest percentage 14(70.0%) for relapsed cases. The distribution of resistant MDR isolates to the second line anti-tuberculous drugs according to the patient's category and type of resistant, the results showed that 2 isolates were pre-XDR which are recovered from relapse and newly diagnosed cases, 2 and 5 isolates were mono-resistant and poly-resistant; all of them were recovered from previously treated PTB cases.

Table 3 showed the results of biochemical tests of the 50 M.TB. isolates obtained throughout the present study. Figure 1 display the patterns of MDR isolates according to the types of first line anti-tuberculous drugs. Drug

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Table 4

Rate of resistant MDR isolates to second line anti-tuberculous drugs

Antituberculous drug	N0. Sensitive isolates (%)	No. resistant isolates (%)
Kanamycin (KA)	18 (90)	2 (10)
Amikacin (AN)	20 (100)	0 (0)
Ethionamide (ETA)	16 (80)	4 (20)
D-cycloserine (CS)	14 (70)	6 (30)
Clarithromycin (CLR)	17 (85)	3 (15)
Ciprofloxacin (CP)	20 (100)	0 (0)
p-amino salicylic acid (PAS)	18 (90)	2 (10)
Rifabutin (RFB)	15 (75)	5 (25)

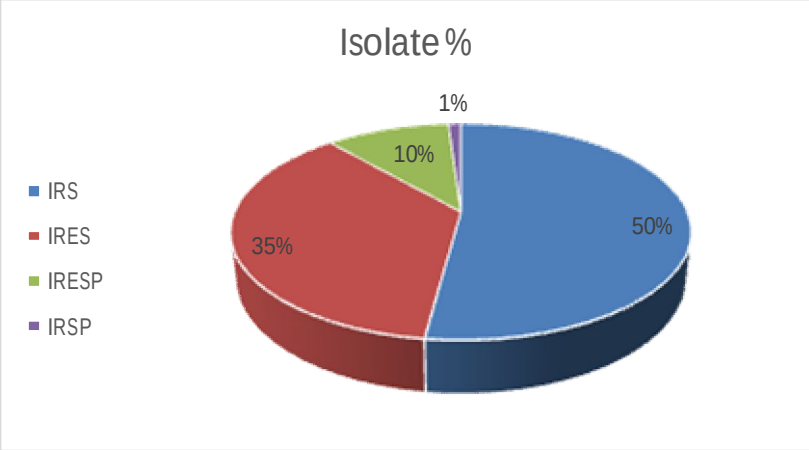

Figure 1

Patterns of MDR isolates according to types of drugs, * I= Isoniazid, R= Rifampicin, S=Streptomycin, E= Ethambutol, P= Pyrazinamid

susceptibility testing for first line anti-tuberculous drugs revealed that 6 out of 50 (12%) isolates were mono-resistant to one of the five drugs; 3 strains were resistant to Rifampicin, 2 strains were resistant to Isoniazid, and one isolatewas resistant to Streptomycin. Additionally, 20 (40%) of the isolates were multi-drug resistant. The overall MDR-TB was recorded in 13.3% of PTB patients. The 20 MDR-M.TB isolates were tested against the second line anti-tuberculous drugs. The results showed that 2 isolates were resistant to Kanamycin, 4 were resistant to Ethionmide, 6 were resistant to D-cycloserine, 3 were resistant to Clarithromycin, 2 were resistant to p-amino salicylic acid, and 5 were resistant to Rifabutin, (Table 4).

4. DISCUSION

The emergence and spread of MDR-TB remains an increasing problem which adversely affects patient care and public health and a major concern of tuberculosis control programs worldwide. The present study found that 40% of *M. tuberculosis* isolates were MDR, and the overall rate of MDR-TB was 13.3% among PTB patients in Diyala province, 10.7% were among previously treated and 2.7% among newly diagnosed PTB cases. The WHO in its 2012 global report on tuberculosis estimates that 3.7% of new cases and 20% of previously treated cases have MDR-TB (WHO, 2012). Accordingly, our findings are within the global range; however, it is higher than the 4.8% rate reported among inmates with PTB in 2001 in Diyala province (SAS). But, it is lower than the 18.8% MDR rate reported in Dohuk province- North Iraq (Merza et al., 2011). What is more astonishing is that in another study using the PCR technique for the detection of drug resistance mutations conducted in Karbala province- South Iraq, 79.7% of M. Tuberculosis isolates were found to be MDR (Mohammed et al., 2013).

The frequency of MDR-TB varies substantially among countries, being about 60% of these cases occurred in Brazil, China, India, the Russian Federation and South Africa alone (Royce, 2013; Lynchet al., 2013; Asaadand Alqahtan, 2012). *M. tuberculosis* develops drug resistance exclusively through chromosomal mutations, in particular single-nucleotide polymorphisms. Moreover, the organism exhibits a spontaneous mutation rate that is at the lower end of thebacterial spectrum. Despite this, whole-genome sequencing technology has identified unexpected genetic diversity among clinical *M. tuberculosis* populations, suggesting that the mycobacterial mutation rate may be modulated within the host and, in turn, implies a potential role for constitutive and/or transient mutated strains in adaptive evolution (Mohammed et al., 2013; Sherman et al., 2011). Ultimately, accumulated evidences affirmed that characteristics of the pathogen itself contributes to this remarkablegenetic variability making *M. tuberculosis* is an extremely successful pathogen that continues to evolve and spread in developing as well as developed countries (Bloom and Small, 1998; Beh, 2013).

It has been documented that crises caused by armed conflict, forced population displacement, or natural disasters result in high rates of excess morbidity and mortality from infectious diseases, such environmental factors were reported to act as key mutagens during *M. tuberculosis* infection (Kimbrough et al., 2012). Undoubtedly, Iraq has exposed to several episodes of armed conflicts, economic blockade and internal and external population

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displacement during the past 20 years ago. Taken together, these factors and its relevant consequences may predispose, at least in part, for the development and emergence of MDR-TB.

Concerning the drug resistance of *M. tuberculosis* isolates to the second line antituberculous drugs, the current study found that 2 out of 20 (10%) MDR isolates had monoresistance to individual drugs, both of which were from relapse cases. Another 5 (25%) isolates had polyresistance, and again all of which were from previously treated cases. Finally, 2 (10%) isolates were pre-XDR, one of them from newly diagnosed case and another one from relapse case. Therefore, the prevalence of pre-XDR among the PTB patients was 1.3%. Of note, XDR-TB has been reported by 84 countries, and the average proportion of MDR-TB cases with XDR-TB is 9% (WHO, 2010). The emergence of XDR-TB strains is a reflection of poor tuberculosis management, and controlling its emergence constitutes an urgent global health reality and a challenge to tuberculosis control activities in all parts of the world, especially in developing countries and those lacking resources and as well as in countries with increasing prevalence of HIV/AIDS (Sharma et al., 2009; Klopper et al., 2013).

Another remarkable finding of our study was that the majority of MTB isolates resistant to the first and second lines antituberculous drugs were recovered from previously treated PTB patients. These results are consistent with other studies and supporting the fact that MDR- and XDR-TB is manmade problem (Chiang et al., 2010; Jain and Dixite, 2010). However, the emergence of MDR-TB in newly notified TB patients is a serious warning signs that MDR-TB is spreading in the community. Of note, new TB patients comprised a median of 54% of the MDR-TB cases (Royce et al., 2013). Although MDR- and XDR-TB had been reported from developed and developing countries around the world (Shah et al., 1993; Ali et al., 2012). However, owing to the persistence security deterioration, insufficient political will, improper implementation of DOTS strategies, low public health education and social stigma, poor patient compliance, unfortunately makes Iraq a fertile environment for the flourish up and spread of such resistant strains of MTB. At the time when world scientific bodies are involved in the dilemma of MDR- and XDR-TB, the *M. tuberculosis* has surprised the world by its new pattern of drug resistance termed "totally resistant" tuberculosis, a term that is given to recognize strain of *M.tuberculosis* which has an *in vitro* resistance to all first and second line of anti-tuberculous drugs (Klopper, 2013; Velayati et al., 2009; Edwardia et al., 2012).

5. CONCLUSION

In conclusion, a standardized approach to MDR TB surveillance and drug susceptibility test for second line antituberculous drugs, coupled with attempts to increase the laboratory capacity across the Iraqi provinces are recommended.

REFERENCES

1. Ali CL, Rambhala N, Al-Shammri AS, Al-Tawfiq JA. Patterns of antituberculous drug resistance in Eastern Saudi Arabia: a 7-year surveillance study from 1/2003 to 6/2010. *J. Epidemiol. Glob. Health*. 2012, 2(1), 57-60
2. Asaad AM, Alqahtani JM. Primary anti-tuberculous drugs resistance of pulmonary tuberculosis in Southwestern Saudi Arabia. *J. Infect. Public. Health.* 2012, 5(4), 281-5
3. BehrMA. Evolution of Mycobacterium tuberculosis. *Adv. Exp.Med. Biol.* 2013, 783, 81-91
4. Bloom BR, Small PM. The evolving relation between human and Mycobacterium tuberculosis. *N. Engl. J. Med.* 1998, 338(10), 677-8
5. Chiang CY, Centis R. Migliori GB. Drug-resistant tuberculosis: past, present, future. *Respirology*, 2010, 15(3), 413-32
6. Dara M, Dadu A, Kremer K, Zaleskis R, Kluge HH. Epidemiology of tuberculosis in WHO European Region and public health response. *Eur. Spine J.* 2013, 22(Suppl 4), 549-55
7. Edwardia ZF, Amale RA, Ajbani KK, Rodriguez C. Totally drug –resistant tuberculosis in India. *Clin Infect Dis*. 2012, 54, 579–81
8. Fuji Ki A. TB Microscopy. The Research Institution of Tuberculosis, Japan. Japan International Cooperation Agency, 1998
9. Grange JM. Mycobacterium. In: Medical Microbiology. A guide to microbial infections; pathogenesis, immunity, laboratory diagnosis and control. By Greenwood D, Slack R, Peutherer J, Barer M. 7th. Ed. 2007, Churchill Livingstone
10. Hasan ASH, Hasan AA, Al-Meshadani JI. Treatment outcomes of inmates with pulmonary tuberculosis in Baquba penitentiary: A follow-up study. *Iraqi J. Comm. Med*. 2008, 21(4), 303-7
11. Jain A, Dixit P. Multidrug resistant to extensively drug resistant tuberculosis: What is next?. *J Biosci.* 2008, 33, 605–16
12. Kent PT, Kubica GP. Public health mycobacteriology: a guide for the level III laboratory, 1985. Atlanta, Ga.: U.S. Department of Health and Human Services/Public Health Service/Centers for Disease Control
13. Khoharo HK, Shaikh IA. Drug resistance patterns in pulmonary tuberculosis. *J. Pak. Med. Assoc.* 2011, 61(3), 229-32
14. Kimbrough W, Saliba V, Dahab M, Haskew C, Checchi F. The burden of tuberculosis in crisis-affected populations: a systematic review. *Lancet Infect. Dis*. 2012, 12(12), 950-65
15. Klopper M, Warren RM, Hayes C, Gey van Pittius NC, Streicher EM, Müller B, Sirgel FA, Chabula-Nxiweni M, Hoosain E, Coetzee, G, David van Helden P, Victor TC, Trollip AP. Emergence and spread of extensively and totallydrug-resistant tuberculosis, South Africa. *Emerg. Infect. Dis*. 2013, 19(3), 449-55
16. Lynch JB. Multidrug-resistant Tuberculosis. *Med. Clin. North Am*. 2013, 97(4), 553-79
17. Merza MA, Farnia P, Salih AM, Masjedi MR, Velayati AA. First insight into the drug resistance pattern of *Mycobacterium tuberculosis*in Dohuk, Iraq: using

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spoligotyping and MIRU-VNTR to characterize multidrug resistant strains. *J. Infect. Public Health*, 2011, 4(1), 41-7
18. Mohammed SH, Ahmed MM, Ahmed AR. First experience with using simple polymerase chain reaction-based methods as an alternative to phenotypic drug susceptibility testing for *Myobacteriumtuberculosis* in Iraq. *Int. J. Appl. Bas Med. Res.,* 2013, 3(2), 98-105
19. Prasad R. Multidrug and extensively drug-resistant TB (M/XDR-TB): problems and solutions. *Indian J. Tuberc*. 2010, 57(4), 180-91
20. Royce S, Falzon D, van Weezenbeek C, Dara M, Hyder K, Hopewell P, Richardson MD, Zignol M. Multidrug resistance in new tuberculosis patients: burden and implications. *Int. J. Tuberc. Lung Dis*. 2013, 17(4), 511-3
21. Shah NS, Pratt R, Armstrong L, Robison V, Castro KG, Cegielski JP. Extensively drug-resistant tuberculosis in the United States, 1993-2007. *JAMA*, 2008, 300(18), 2153-60
22. Sharma SK, George N, Kadhiravan T, Saha PK, Mishra HK, Hanif M. Prevalence of extensively drug-resistant tuberculosis among patients with multidrug-resistant tuberculosis: a retrospective hospital-based study. *Indian J. Med. Res.* 2009, 130(4), 392-5
23. Sherman DR, Gagneux S. Estimating the mutation rate of Mycobacterium tuberculosis during infection. *Nat. Genet*. 2011, 43(5), 400-1
24. Statistical Analysis System (Thomas). User's Guide Statistical. Version 17th Ed. Inst. Inc, Cary. N.C. USA
25. Thomas L. Clinical Laboratory Diagnostics. Use and assessment of clinical laboratory results. 1st. Ed. 1998. TH-Books, Germany
26. Velayati AA, Masjedi MR, Farnia P, Tabarsi P, Ghanavi J, Ziazarifi AH, Hoffner SE. Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. Chest, 2009, 136(2), 420-5
27. World Health Organization, Global tuberculosis report 2012, Geneva, Switzerland
28. World Health Organization. Extensively drug-resistant tuberculosis (XDR-TB): recommendations for prevention and control. *Wkly Epidemiol Rec.* 2006, 81, 430-2
29. World Health Organization. Multidrug and Extensively Drug-resistant Tb (M/XDR-TB): 2010 Global report on surveillance and response (WHO/HTM/TB/2010.3)
30. World Health Organization. WHO report on the tuberculosis epidemic, Geneva, WHO, 1997

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