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 study chosen by random selection. Sputum and broncho-alveolar washing samples were collected and submitted to direct Ziehl-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 were 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 resistant to one of the second line drugs. 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 resistant tuberculosis, 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 TB caused by 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 primary causes of MDR-TB are inappropriate treatment, inappropriate or incorrect use of anti-TB drugs, or use of poor quality medicines (Jain and 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 broncho-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 Ziehl-Neelsen stain (Fuji Ki, 1998). Thereafter, each specimen was cultured on slants of Lowenstein-Jenson and slants of Stonebrick media in duplicate (Grange and Mycobacterium, 2007). Cultures were incubated in 37°C 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 (9). 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
(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 MDR isolate.

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 were follow-up, 20 were relapse, and 32 chronic cases, besides 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
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 isolate was 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 Ethionamide, 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. DISCUSSION
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 the bacterial 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 remarkable genetic 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...
displacement during the past 20 years. 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 a well-known 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 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