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First insight into the drug resistance pattern of *Mycobacterium tuberculosis* in Dohuk, Iraq: Using spoligotyping and MIRU-VNTR to characterize multidrug resistant strains

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Summary The objectives of this study were to determine drug resistance pattern in new and previously treated tuberculosis (TB) patients, to assess function of TB control program, and to characterize multidrug resistant TB (MDR-TB) by molecular fingerprinting methods. Anti-micorbial susceptibility testing (AST) to the first line anti-TB drugs was performed on Löwenstein—Jensen (middlebrook 7H10) medium according to the proportion method. Molecular fingerprinting of all MDR strains was performed by spoligotyping and MIRU-VNTR. *Mycobacterium tuberculosis* strains were isolated from 53 Iraqi patients with pulmonary TB. Thirty eight patients (71.7%) tested cases, and 15 (28.3%) were previously treated. Four of the 38 new cases (10.5%) had resistant, 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. Spoligotyping of MDR strains showed CAS family (40%) as the predominant genotype. Using MIRU-VNTR typing, all isolates had a unique profile. MDR-TB prevalence is higher among previously treated patients than among the new cases. The many drug resistant strains, in absence of evidence of recent transmission and in combination with the many previously treated cases, highlight the need for an improved control program, coupled with a need to improve detection rate and early diagnosis of MDR-TB.

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Introduction

Despite the adoption of Directly Observed Therapy Short-Course (DOTS) strategy by World Health Organization (WHO), TB prevalence continues to increase worldwide, particularly in developing countries. This has been attributed to the human immunodeficiency virus (HIV) pandemic and emergence of drug resistant strains [1]. Additional reasons for the increase are related to poverty, migration, ethnic conflicts and abuse of narcotic substances. Of great importance are also inadequate health care generally attributed to poor performance of NTPS. These factors may lead to the development of drug resistance, which however, can be counteracted by proper health control measures [2]. The emergence of MDR-TB, that is *Mycobacterium tuberculosis* strains resistant to at least isoniazid (INH), rifampicin (RMP), poses a significant global and public health concern. Such cases are difficult to cure, requiring the use of second line drugs that are more toxic and expensive than the first line regimen [3]. The recently published WHO report on Global TB Control in 2009 stated that there were an estimated 0.5 million cases of MDR-TB in 2007 [1]. Recently, *M. tuberculosis* strains that are extensively drug resistant-TB (XDR-TB), i.e. MDR-TB strains resistant to at least three of the six classes of second-line drugs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and para-aminosalicylic acid) have been described [4,5]. According to a 2000—2004 survey of International TB Laboratories conducted by the WHO and US Centers for Disease Control (CDC), 20% of *M. tuberculosis* isolates were MDR-TB and 2% were XDR-TB [6]. Anti-TB drug resistance is present everywhere in the world and MDR-TB is considered to be widespread today. A high prevalence of MDR-TB cases has been noticed in China, India, and the Russian Federation. China and India carry approximately 50% of the global burden and the Russian Federation a further 7% [7].

Iraq is one of the countries in WHO Eastern Mediterranean Region (WHO-EMR) with a relatively high TB incidence rate (56/100,000) and low case detection rate (45%) [1]. This may be attributed to the unstable situation in society, low socio-economic status, and unsatisfactory treatment. The DOTS strategy has been adopted in Iraq since 1998, except for the three northern Iraq provinces (Dohuk, Erbil and Sulaimani), which implemented DOTS since 2001. In northern Iraq, the management of TB patients is based primarily on smear microscopic examination as there is no culture and antimicrobial susceptibility testing (AST) [8]. In case of treatment failure or suspicion of drug resistance, patients are referred to Baghdad or neighbouring countries for further investigations. The DNA fingerprinting methods have been widely used to characterize drug resistance strains, particularly MDR-TB, in order to better understand the origin and propogation of drug resistant strains in a specific location. The first effective strains genotyping technique, RFLP-IS*6110*, has gained recognition as the international standard for epidemiological typing of *M. Tuberculosis* [9]. Recently, spoligotyping, a highly polymorphic direct repeat (DR) locus in the MTB genome has been used to provide epidemiological information of strain relatedness [10]. More recently, mycobacterial interspersed repetitive unit-variable number of tandem repeats (MIRU-VNTR) analysis have been established to track epidemic strains [11].

Because the TB drug resistance patterns and genotype characterization of resistant strains are currently unknown in Dohuk, the goal of the presents study was to determine drug resistance pattern in new and previously treated TB patients, to evaluate TB control programs, to determine risk factors associated with MDR-TB and finally to characterize MDR strains by molecular fingerprinting methods.

Materials and methods

Specimen collection

All smear positive pulmonary specimens were collected between June 2008 and June 2009 at the NTP center of Dohuk province — Iraq. Sampling was deemed to be representative at the province level, as all TB cases had attended directly or had been referred from other health centers to the Dohuk NTP. Classical epidemiology data were collected by using standard questionnaires. Information was obtained on sex, age, country of birth, recent smear positivity, previous history of TB, and present address. The specimens were shipped to Mycobacteriology Research Center (MRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Tehran, Iran for culturing and DST.

Isolation of *M. tuberculosis*

The pulmonary specimens were processed for culture by digestion, decontamination and concen-