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Snapshot of the genetic diversity of *Mycobacterium tuberculosis* isolates in Iraq

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ABSTRACT

This study explored the genetic diversity of *Mycobacterium tuberculosis* isolates in Iraq by spoligotyping and 15-locus-based mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) methods. Initially, 270 isolates from 134 patients were collected and then 134 non-duplicating isolates (1 isolate/patient) were subjected to the study analyses, 70 isolates were found to be multidrug resistant (MDR) upon testing by proportion method on Löwenstein–Jensen medium.

Spoligotyping yielded 39 patterns; 111/134 (82.2%) isolates being grouped in 16 clusters vs. 23/134 (17.2%) isolates being unique. SIT1144/T1 represented the largest cluster ($n = 20$, 14.9%), followed by SIT25/CAS1_Delhi ($n = 19$, 14.2), SIT22/CAS1_Delhi ($n = 12$, 9%); the other clusters ranged from 2 to 8 isolates. The SIT1144 is not reported in neighboring countries and only 4 isolates were reported worldwide (2 in USA, 1 in Venezuela, and 1 in Greece). This study reported 4 isolates belonging to SIT41/Turkey family, and thus it seems that this family is not exclusive to Turkey as previously thought. CAS lineage was predominant in this study (42.5%), followed by ill-defined T (29.9%).

Highly diverse MIRU-VNTR genotypes were displayed; 100 distinct MIRU-VNTR genotypes were detected (8 clusters with 2–8 strains/cluster and 92 unique). The clustering rate was 18.03%. The discriminatory efficiency of MIRU-VNTR was high (Hunter-Gaston discriminatory index [HGDI] = 0.992); it was higher than that of spoligotyping (HGDI; 0.930). However, the highest discriminatory power was provided by spoligotyping and MIRUs together. Owing to the low clustering rate by MIRU-VNTR, these results suggest that drug-resistance TB in Iraq is due to acquired resistance as opposed to transmission.

Conclusion: Iraq is specific in having its own most predominant lineage (SIT1144/T1) which is not found among neighboring countries. The 15-locus MIRU-VNTR can be useful in discriminating *M. tuberculosis* isolates in Iraq.

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Introduction

Infection with *Mycobacterium tuberculosis* is responsible for extensive morbidity and mortality worldwide with approximately 8.8 million new cases and 1.1 million deaths reported in the year 2010 [1]. The importance of TB as a major public health problem has dramatically escalated with the emergence of the multi-drug resistant tuberculosis (MDR-TB), defined as a combined resistance to Rifampicin and Isoniazid [2].

Iraq is one of the countries with the highest incidence among the Eastern Mediterranean region with TB accounting for 56/100,000 population, and the estimated MDR-TB cases among new pulmonary TB cases notified in 2010 is 210 (50–380), whereas the estimated number among retreated pulmonary cases is 160 (57–260) [3]. These data are being extracted from the cases registration system that is based on clinical findings and microscopy. In the last few decades, Iraq has witnessed political instabilities and consecutive wars that left their marks on the health system. Unfortunately, there is no reliable study yet conducted to figure out the impact of those conditions on TB epidemiology in this country. Although genotyping of *M. tuberculosis* is currently appreciated as a significant complement for TB control programs [4–8], very limited data are available on the genotypes of MTB strains circulating in Iraq [9]. In contrast, in the last few years, several studies were published describing the genotypes of MTB strains isolated in neighboring countries such as Turkey, Iran and Saudi Arabia [10–15].

This study sought to conduct a molecular study to explore the genetic diversity and *M. tuberculosis* isolates in Iraq. Its aim was to shed some light on the population structure of MTB strains in Iraq and its comparison with that in the neighboring countries. It is believed that conducting such a study would generate a sort of data that may eventually lead to a better understanding of tuberculosis epidemiology in Iraq, especially the transmission dynamic of this microorganism. Although *IS6110*-RFLP has been considered the golden standard for studying the molecular epidemiology of *M. tuberculosis*, its use has been limited [16]. Alternatively, use of PCR-based genotyping methods like spoligotyping and Mycobacterial Interspersed Repetitive Units-Variable Tandem Repeats (MIRU-VNTR) have gained wide appreciation due to simplicity, rapid availability of results, reproducibility and possibility of digitizing data and exchange for inter-laboratories communications and comparisons [17].

In this study it is believed that spoligotyping and MIRU-VNTR genotyping methods present the largest amount of molecular epidemiology data on *M. tuberculosis* from Iraq to date.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of the Iraqi Ministry of Health and was performed in accordance with all national regulations. Nonetheless, since the mycobacterial isolates were collected from patients' routine samples, this study was considered a laboratory study and ethics approval was not required. Although it was a retrospective study, anonymity of the patients were maintained through using a special coding system based on numbers to ensure that this study would have no chance to affect the patient's welfare.

Mycobacterial clinical isolates and DNA extracts

During the period from January 2011 to July 2012, initially all culture positive *M. tuberculosis* isolates (more than 270 isolates) were collected and isolated from Iraqi patients with active pulmonary tuberculosis that attended the National Reference Laboratory of the National Center of Tuberculosis and chest illnesses (NTP – Iraq). Because of that this laboratory is the only lab across Iraq accredited with performing culture and drug susceptibility testing; there was a referral bias towards treatment failure. The isolates were identified as *M. tuberculosis* according to standard phenotypic criteria. Testing of drug susceptibility of these isolates to four first-line anti-TB drugs (Rifampin, Isoniazid, Streptomycin, and Ethambutol) was performed by agar proportional method on Lowenstein–Jensen media as described elsewhere (WHO, 2008). Among the 270 isolates, there were multiple isolates from the same patients, recovered on different visits to the laboratory. As all of the multiple isolates/patients gave the same profile on Spoligotyping and MIRU-VNTR, one isolate per patient was selected. Thus, 134 non-duplicated *M. tuberculosis* culture isolates (1 isolate/patient) were obtained that were subjected for the study analyses later on. Mycobacterial genomic DNA was extracted from cultured cells as described previously (13, 14, 15).

Genotyping of the isolates

The isolates were characterized by two genotyping methods, spoligotyping and MIRU-VNTR. Spoligotyping was performed as previously described [18]. MIRU-VNTR genotyping was performed by PCR-amplification of a panel of 15 MTB MIRU loci using primers described in the MIRU-VNTR standard protocol [17]. *Mycobacterium bovis* P3 and H37Rv were used as positive controls and autoclaved with double distilled water as a negative control. The results were compared with updates of the international spoligotype database of the Pasteur Institute of Guadeloupe which provides information on the shared-type distribution of *M. tuberculosis* spoligotypes at the worldwide level [18]. At the time of the comparison, the updated SITVIT2 version contained more than 90,000 patterns from more than 160 countries of patient origin.

MIRU-VNTR profile matching a pre-existing profile was classified as MIRU-VNTR international type (MIT). Newly created MIT results either after matching of an orphan profile in the study with another orphan profile present in the database or when two or more isolates within the study had the same new MIRU-VNTR profile. Additionally, MIRU-VNTR clusters arise when two or more isolates within the study had an identical profile.