Current Research in Microbiology and Biotechnology
Vol. 5, No. 6 (2017): 1350-1357
Research Article
Open Access

ISSN: 2320-2246

Spoligotyping of Pulmonary Tuberculosis in Iraq

Shatha Thanoon Ahmed[1,*], Ruqaya Mustafa Ali[2], Ahmed Asmer Mankhi[3] and Batool Ali Shihab[1]

[1] *University of Baghdad, Science College for women, Dept. of Biology, Iraq.*
[2] *Ministry of Agriculture, Veterinary Department, Baghdad, Iraq.*
[3] *Ministry of Health, Chest and Respiratory Disease Center, Baghdad, Iraq.*

* Corresponding author: Shatha Thanoon Ahmed; e-mail: shatha_thanoon@yahoo.com

Received: 11 September 2017 Accepted: 22 September 2017 Online: 25 November 2017

ABSTRACT

Tuberculosis (TB) is an infectious disease which is caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), remains a health problem in Iraq especially Multi Drug Resistant Tuberculosis (MDR-TB) like many other parts of the world. The present study was the first study applying spoligotyping in Specialized Chest and Respiratory Disease Center/ National Reference Laboratory for Tuberculosis (NRL) in Baghdad. A total of 77 MDR-TB isolates the results showed that 2(2.6%) isolates given negative and excluded from our study, the result of 75 isolates showed 11 lineages and 6 families among tuberculosis strains, the most predominant lineage was Delhi / CAS (36%) followed by TUR and Ghana lineage (20%), while the T and Central Asian (CAS) were the main spoligotype families with (30.7% and 29.3%) respectively. Spoligotyping yielded 36 patterns, 49 isolates grouped in 10 clusters (clustering rate 65.3%, ranging from 2 to 14 strains per cluster) and 26 isolates displayed unclustered or unique pattens, 16 of these 26 unique patterns corresponded to orphan patterns in the international multi marker database SITVIT2.The Spoligotype International Type 1144 (SIT1144/T1) represented the largest cluster (n=14,18.6%). Comparison with the SITVIT2 database showed that Iraq is unique in having its own most predominant strain which is not reported in neighboring countries and only 4 isolates were reported worldwide. This study also reported two strains which are globally rare (SIT309 and SIT1916). Three 3(4%) strains were reported as new genotypes or unknown strain suggested that these strains might be transmitted from these regions to our country. In conclusion, this study showed that the genotyping of *M. tuberculosis* is important in the identification of related strains and characteristic disease phenotypes.

Keywords: *M. tuberculosis*, MDR-TB, spoligotyping, SIT1144.

1. INTRODUCTION

Although MDR-TB is still a public health, problem in Iraq, there is little information about the genetic characteristics of the isolates causes the epidemic. Analysis of genetic polymorphism, among drug resistant of *M. tuberculosis* strains may help provide some vision into the transmission dynamics of these strains in the country and can control the epidemic [1]. Spoligotyping (spacer-oligonucleotide typing), widely used technique for simultaneous detection and typing of *M. tuberculosis* complex, based on 43,known spacers,interspersed between, direct repeat regions(DR). Results can be obtained within 1 day. Thus, the clinical usefulness of spoligotyping is determined by its rapidity, both in detecting causative bacteria and in providing, epidemiologic information on strain, identities [2].

Numerous studies have been carried out to determine, the association of drug resistance, with spoligotype defined lineages; much data has been produced over the years. New information continues to be generated [1]. This study aims to understand the correlation of drug resistance with,lineages of MDR-TB isolates. This would help provide a perspective of the, available data that can be used as a starting point to understand, the molecular epidemiology of drug resistant TB.

2. MATERIALS AND METHODS

2.1 Clinical isolates

Seventy seven MDR-TB isolates were selected from sputum samples collected from patients that attended to Specialized Chest and Respiratory Disease, Center/ National Reference Laboratory for Tuberculosis, (NRL) in Baghdad. This study was approved by the local ethical committee.

2.2 DNA extraction

Genomic DNA was extracted from isolates by sonification according to [3].

2.3 Genotyping of the isolates

MDR isolates were characterized by genotyping Method (spoligotyping). Spoligotyping analysis was performed by using commercial kit (Mapmygenome / India) as described by [4] .The 43 spacers between the direct repeats in the target region were amplified by using DRa biotinylated and DRb primers. The PCR products were hybridized to a membrane containing 43 oligonucleotides by reverse line blotting. *M. tuberculosis* H37Rv and *M. bovis* BCG were used as positive controls in each run. The results were analyzed by using the SITVIT2 proprietary database of the Pasteur Institute of Guadeloupe, which is an updated version of the previously SpolDB4 and SITVITWEB databases. It provides information on the shared-type, distribution of *M. tuberculosis* spoligotypes at the worldwide level [2, 5].

3. RESULTS AND DISCUSSION

A total of 77 MDR-TB isolates the results showed that 2(2.6%) isolates given negative and excluded from our study, the data were available for 75 (97.4%) strains. The results showed that these strains belong to 11 lineages: Delhi / CAS was the most prevalent lineage 27(36%), followed by TUR and Ghana were 15(20%) to each lineage, Haarlem 5(6.67%), NEW-1, Uganda II, S and EAI were 2(2.67%) to each lineage, Cameroon and LAM were found as single type (1.33%). Remaining 3(4%) strains could not be identified because they were not among those that were available in the MIRU-VNTR *plus* database and classified as unknown of genotypes therefore could be new (Table 1). A total of 75 MDR strains, 59 (78.7%) strains were represented in SpolDB4 and had previously defined shared type number (SIT). These strains consisting of 20 different patterns belonging to 6 families included: CAS with sub-family CAS and CAS1; H with sub- family H3 and H4; T with sub-family T1 and T3, S family, LAM with sub-family LAM9 and MANU with sub family MANU2. Among 75 MDR strains, T and CAS family were the main spoligotype families 23(30.7%) and 22(29.3%) strains respectively, followed by MANU family in 6 (8%) strains, H family in 5(6.7%) strains, S family in 2(2.7%) strains and LAM family was represented in a single strain (1.3%) (Table 2).

Table 1: Distribution of spoligotype lineages for 75 MDR-TB strains.

Spoligotype Lineage	No. of isolates	Percentage %
1) Delhi/CAS	27	36
2) TUR	15	20
3) Ghana	15	20
4) Haarlem	5	6.67
5) NEW-1	2	2.67
6) UgandaI	2	2.67
7) S	2	2.67
8) EAI	2	2.67
9) Cameroon	1	1.33
10) LAM	1	1.33
11) Unknown	3	4

The remaining 16 strains (21.3%), representing 16 unique spoligotype patterns that could not be classified under any of the shared types in the SpolDB4 database, thus were defined as (orphan). These unidentified spoligotypes were analyzed by MIRU-VNTR plus. Among these orphan, 5 strains were classified with Delhi/CAS linage, 2strains were classified with Ghana, Haarlem and EAI lineage, while single strain was classified with TUR and Cameron lineage. The remaining 3 orphan spoligotypes were not found in the database and did not show similarity to any other known lineages therefore, were considered as new genotypes or (unknown). Table 3 shows the spoligotype description of 16 orphan isolates obtained in this study. Figure 1 shows the spoligopatterns of some of samples tested.

3.1 Spoligotyping cluster

The dendrogram was generated by using the Unweighted Pair Group method with Arithmetic Averages (UPGMA) algorithm based on the MIRU-VNTR data. The UPGMA tree of *M. tuberculosis* genotypes is illustrated in Figure 2. Among 75 MDR- TB isolates, 49 (65.3%) isolates were grouped in 10 clusters (2to 14 isolates per cluster) while 26(34.7%) isolates displayed unclustered or unique spoligotype spatterns (1 isolate only), Sixteen of these 26 unique patterns corresponded to orphan patterns in the SITVIT2 database, thus there were 36 patterns. The distribution of 10 clusters with known SIT numbers is shown in table 4. The size of the clusters ranged from 2 to 14 strains per cluster, the largest cluster was SIT 1144 (14strains, 18.6%), SIT 26 represented the second

largest cluster (9 strains, 12%) while the third largest cluster was SIT54 (6 strains, 8%). Other clusters were SIT25 (5 strains, 6.6%), SIT53 (4 strains, 5.3%) and SIT50 (3 strains, 4%). There were four clusters of two strains 2.7% (SIT22, SIT34, SIT127 and SIT1198).

3.2 Clustering and transmission rates

Clustering is a marker for recent or ongoing transmission, in population while unique pattern are associated, with reactivation of infection [6]. In present study, spoligotyping of 75 MDR isolates yielded clustering rates 65.3% (distributing 49 strains in 10 clusters) and by using formula from [6] the recent transmission rate was calculated as 52%. We also showed a diversity (D) among the isolates which was calculated, by dividing the number of different patterns by the number of isolates analyzed. The degree of diversity was found 48 (D = 36/75 × 100).

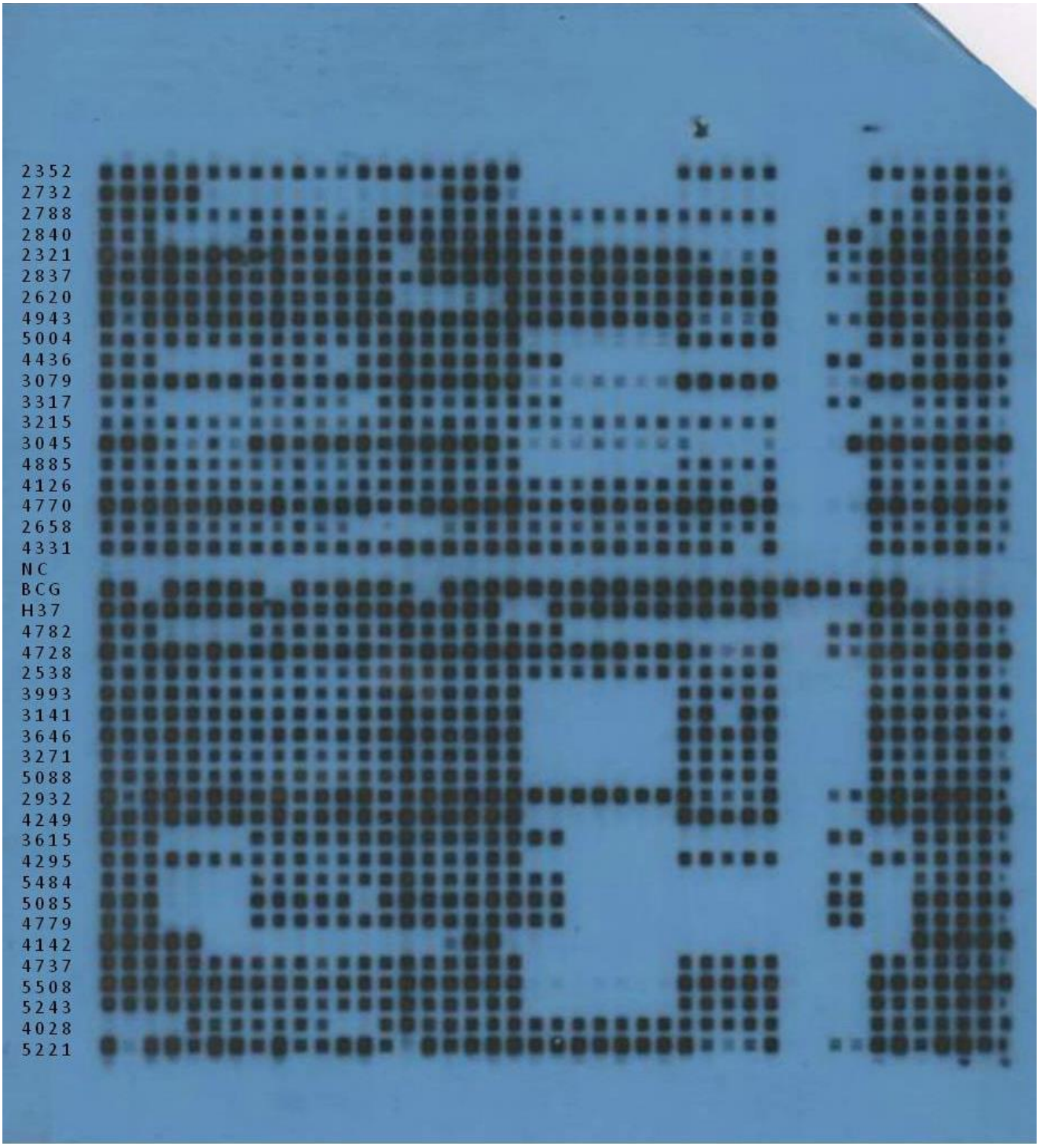

Figure 1: Spoligotype autoradiograph of some MDR-TB isolates tested.

Patient's number followed by spoligotype pattern of 40 MDR-TB strains with negative control (NC) and two positive controls *M. bovis* BCG strain (absence of spacers 3, 9,16 and 39-43) and H37Rv strain (absence of spacers 20–21 and 33-36). In the 43 spacers, Black square reveals the presence of a spacer and a blank one its absence.

Table 2: Spoligotypes represented among MDR-TB strains with SIT (n=59).

Spoligotype patterns(binary format)	SIT	Octal format	No.	lineage	Sub-family	Family
[redacted]	37	777737777760771	1	Ghana	T3	T
[redacted]	53	777777777760771	4	Ghana	T1	
[redacted]	102	777703777760771	1	UgandaII	T1	
[redacted]	205	737777777760771	1	Ghana	T1	
[redacted]	284	037637777760771	1	Ghana	T1	
[redacted]	1144	777777600760771	14	TUR	T1	
[redacted]	1916	777701777760771	1	UgandaII	T1	
[redacted]	22	703777400001771	2	Delhi/CAS	CAS	CAS
[redacted]	356	703777600001771	1	Delhi/CAS	CAS	
[redacted]	25	703777740003171	5	Delhi/CAS	CAS1_ DELHI	
[redacted]	26	703777740003771	9	Delhi/CAS	CAS1_ DELHI	
[redacted]	247	703777740003471	1	Delhi/CAS	CAS1_ DELHI	
[redacted]	309	703767740003171	1	Delhi/CAS	CAS1_ DELHI	
[redacted]	428	703777740003371	1	Delhi/CAS	CAS1_ DELHI	
[redacted]	1198	703737740003171	2	Delhi/CAS	CAS1_ DELHI	
[redacted]	54	777777777763771	6	Ghana	MANU2	MANU
[redacted]	50	777777777720771	3	Haarlem	H3	H
[redacted]	127	577777777420771	2	NEW-1	H4	
[redacted]	34	776377777760771	2	S	S	S
[redacted]	42	777777607760771	1	LAM	LAM9	LAM

Table 3: Spoligotypes of orphan strains among MDR-TB strains without SIT (n=16).

Binary spoligotype pattern	NO.	Octal code	Lineage
[redacted]	1	703777700003171	Delhi/CAS
[redacted]	1	703777600003171	Delhi/CAS
[redacted]	1	703677740003171	Delhi/CAS
[redacted]	1	703577740003771	Delhi/CAS
[redacted]	1	501767740003471	Delhi/CAS
[redacted]	1	557767777763771	Ghana
[redacted]	1	777767777663771	Ghana
[redacted]	1	037637777720771	Haarlem
[redacted]	1	777617777720771	Haarlem
[redacted]	1	777777777423771	EAI
[redacted]	1	777771777423771	EAI
[redacted]	1	777777600660771	TUR
[redacted]	1	760003400000171	Cameron
[redacted]	1	760003600000171	-----
[redacted]	1	777777777421771	----
[redacted]	1	037601777760771	----

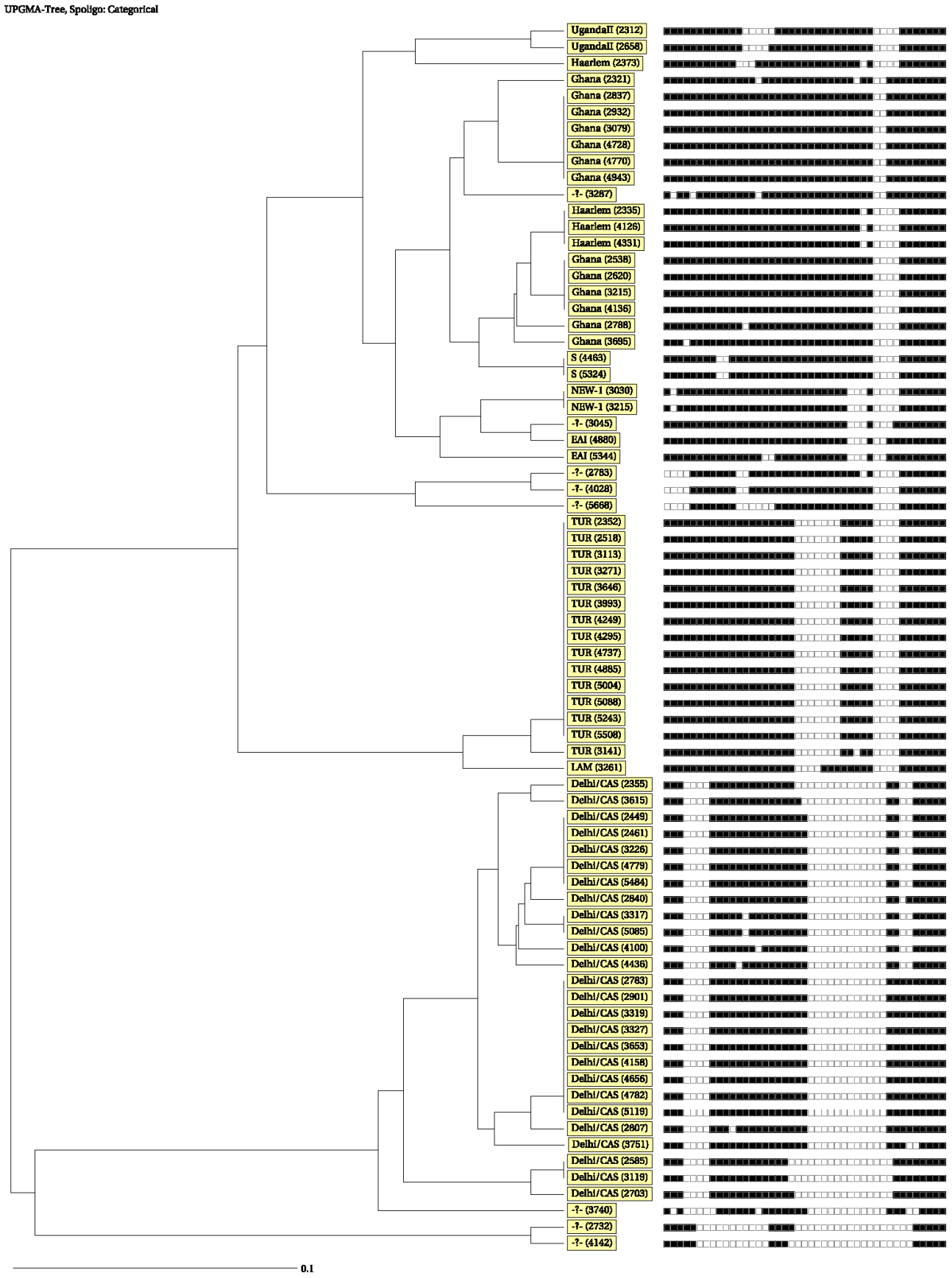

Figure 2: Dendrogram of 75 MDR-TB isolates. Clustered patterns (n =49 strains in 10 clusters), clustered strains correspond to a similar spoligotype pattern shared by 2 or more strains. Unique patterns (n=26 strains), unique strains harboring a spoligotype pattern that does not match with another strain from this study .The yellow column shows the lineage followed by the patient's number in this study). Clustered using the unweighted pair group method using arithmetic averages (UPGMA) in MIRU-VNTR*plus* software.

The predominant lineage in this study was the Delhi/CAS (36%). In the previous reports, this lineage has been shown as a predominant strain in the central Asian countries where TB is endemic such as Afghanistan and Pakistan [7] and India [8]. This result was agreed with study in Baghdad by [9] which found the CAS Lineage strains formed the biggest (31.4%) lineage of strains infecting Iraqi patients. Moreover, this lineage has also been identified as predominant lineage in two neighboring countries to Iraq such as Saudi Arabia [10] and Iran [11] which shares long geographical, borders with Iraq.

In this study, the ill-defined T and CAS was most predominant family in our study (23strains, 30.7%) and (22 strains, 29.3%) respectively. T family is corresponds to about 30% of all entries in the international database [2]. In addition, this family was dominant in the two largest surrounding countries to Iraq (Iran and Turkey) [12] and also was the major family circulating in the Syrian population [13]. Because Iran and Turkey are known to share, borders and historic links with European countries therefore, it may be suggested that the T–family may have first spread in both country and then transmitted to Iraq as well as Syria.

Two clusters belonging to the T family were reported in this study: first, SIT1144/T1 was the biggest cluster amongst the MDR isolates (n=14, 18.6%). Iraq is unique in having this strain, which is not found among three neighboring countries (Iran, Saudi Arabia and Turkey). The distribution pattern of SIT1144 in SITVIT2 database showed that this strain was reported in USA (n=2), Greece and Venezuela (n=1). Because of geographic distribution, of this strain that were isolated from different regions in Iraq, thus it suggested that the ability of SIT1144 strains to transmit and cause disease was over than other strains.

Table 4: Distribution of 10 clusters with previously described SIT number among 75 MDR strains

SIT	No. of isolates	Lineage	Family	%
SIT1144	14	TUR	T1	18.6
SIT26	9	Delhi/CAS	CAS1_DELHI	12
SIT54	6	Ghana	MANU2	8
SIT 25	5	Delhi/CAS	CAS1_DELHI	6.6
SIT53	4	Ghana	T1	5.3
SIT50	3	Haarlem	H3	4
SIT22	2	Delhi/CAS	CAS	2.7
SIT34	2	S	S	2.7
SIT127	2	NEW-1	H4	2.7
SIT1198	2	Delhi/CAS	CAS1_DELHI	2.7

The second clusters of T family was SIT53 (n=4, 5.3%). In the updated SITVIT2 database this strain is widespread throughout the world and about 21% of this strain was reported in the US. The study by [14] in South Africa found the prevalence rate of this strain was 8% with MDR and 4% with XDR isolates while in another study the rate was 6.1% with MDR strains in China [15]. SIT53 also formed the largest cluster of T family in Iran [11] and according to several spoligotyping studies carried in Iraq [9, 16], in Syria [13] and in Turkey [17] found the T family especially SIT53 (in Turkey) was demonstrated as the major shared type. This correlation suggests a possible role for war, migration, trade and tourism in distribution the strains of T family among these countries.

Four clusters belonging to the CAS lineage were reported in this study. These clusters were of variable sizes, ranging from 9 isolates in SIT26, 5 isolates in SIT 25 to 2 isolates in SIT22 and SIT1198. The presence of the CAS lineage in big and several clusters may indicate successful circulation of this lineage within the population. The SIT26 was the first largest clusters (n=9,12%) of CAS family , The distribution pattern of SIT26 /CAS in SITVIT2 database showed the high prevalence of this strain in US followed by India, Pakistan , Bangladesh and Saudi Arabia . Our results although disagreed with those studies performed by [11] and [18] who reported that CAS family was the most prevalent family than T family in Iran but are agreed with them when reported that SIT26 was the first largest cluster of this family.

MANU family with the cluster SIT54/MANU2 was the third cluster reported in Iraq (n=6, 8%). In fact, the MANU2 family has a low prevalence worldwide, so there are few reports of MANU2 genotype. Previously studies reported a high proportion of MDR isolates within ST54/ MANU2 genotypes (15.1%) in China [15] while another study in Iran reported low rate 4% of this genotype [18]. The Haarlem lineage has been reported by many countrie ssuch asIran, Armenia, Georgia, Finland, and Russia [5]. In Turkey [17] demonstrated that both T and Haarlem families with high phylogeographical specify for Turkey among MDR isolates. It was indicated that this genotype has an epidemiological potential to be transmitted and disseminated throughout the world. Global MDR-TB outbreaks have been associated with Beijing and Haarlem lineages [19]. In the updated SITVIT2 database and looking at the spread of SITs belonging to H families in the world, it can be noted that more than quarter(26.8%) of SIT50/H3family was significantly prevalent in the US followed by Austria, Italy and also in two neighboring countries to Iraq (Saudi Arabia and Turkey).

Ten spoligotypes patterns were non clustered and found as unique patterns or unique strain (n=1,1.3%) as shown in table (2) . according to the update SITVIT2 database, two of these strains (SIT309 and SIT1916) are globally rare , SIT309 is limited to Central African Republic(n=2) and Saudi Arabia(n=1) while the SIT1916 is limited to Turkey(n=3), Italy and Senegal (n=1)and this genotype was not reported in previous study in Iraq by [9]. Other strains such as SIT 205(in US) and SIT 356(in South Africa) were also not reported previously in Iraq. Remaining 6 strains SIT37 (in Venezuela and Saudi Arabia), ST284 (prevalent in Turkey, Saudi Arabia and Bulgaria), SIT247, SIT428, and SIT102 and SIT 42 that are prevalent in the world mainly in the US were reported previously in Iraq in 2013. Unknown genotypes 3 (4%) were spreading in Iraqi patients; these genotypes could not match with any (Lineage specific).

Although, the rate of clustering was much higher than the rate observed in Iraq by [16] and by [9] (20% and 33.3%) respectively, this proportion of clustered strains was in the range with those reported by neighboring countries i.e. Saudi Arabia 86.4% [20], Iran 60.5% [21] and Turkey 79% [17]. High rate of clustering have been attributed to recent infection whereas the low rate is associated with reactivation of a previous TB infection [6].

The degree of diversity was closer to the rates reported in Iran but much lower than rate in Saudi Arabia, the high incidence of clustering Indicates both a high rate of diversity and probable active transmission of TB, this diversity could be attributed to human travel, tourism especially for religious purposes and influx of visitors due to existence of high communication as well as to migration of people from countries where TB is endemic [20].

4. CONCLUSION

Iraq is specific in having its, own most predominant lineage (SIT1144/T1) which is not found among neighboring countries, in addition, most predominance of spoligotypes is similar to the neighboring countries and also to the USA. Other strains were in contrast to some surrounding countries where they did not find these strains but the identified these strains in Iraq have been found in many different countries suggesting that these strains might be circulating worldwide.

5. REFERENCES

1. Panwalkar, N.; Chauhan, D. S.; Desikan, P. (2017). Spoligotype defined lineages of Mycobacterium tuberculosis and drug resistance: Merely a casual correlation. Indian. J. Med. Microbiol. 35(1):27-32.
2. Brudey, K.; Driscoll, J.; Rigouts, L.; Prodinger, W.; Gori, A., et al. (2006). Mycobacterium tuberculosis complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4)for classification, population genetics, and epidemiology. BMC Microbiol. 6:23.
3. Mankhi, A.A. (2015). Manual of Laboratory guide line in the national reference laboratory in Iraq. Ministry of Health. Chest and respiratory Diseases center in collaboration with WHO. Baghdad. Iraq.
4. Kamerbeek, J.; Schouls, L.; Kolk, A.; van Agterveld, M.; van Soolingen, D.; Kuijper, S.; Bunschoten, A.; Molhuizen, H.; Shaw, R.; Goyal, M. and van Embden, J.(1997). Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J. Clin. Microbiol. 35(4):907-914.
5. Demay, C.; Liens, B.; Burguière, T.; Hill , V,; Couvin, D.; Millet, J.; Mokrousov, I.; Sola, C.; Zozio, T. and Rastogi, N. (2012). SITVITWEB - a publicly available international multimarker database for studying Mycobacterium tuberculosis genetic diversity and molecular epidemiology. Infect. Genet. Evol. 12(4):755–766.
6. Small, P.M.; Hopewell, P.C.; Singh, S.P., Paz, A.; Parsonnet, J.; Ruston, D.C. Schecter, G.F.; Daley, C.L.and Schoolnik, G.K., (1994) The epidemiology of tuberculosis in San Francisco. A population -based study using conventional and molecular methods. N. Engl. J. Med. 330: 1703-1709.
7. Hasan, Z.; Tanveer, M.; Kanji, A.; Hasan, Q.; Ghebremichael, S.and Hasan, R. (2006). Spoligotyping of Mycobacterium tuberculosis isolates from Pakistan reveals predominance of Central Asian Strain 1 and Beijing isolates. J. Clin. Microbiol. 44(5):1763-68.
8. Singh, U. ; Suresh, N.; Arora,J.; Pant,H.; Sinha, S.; Aggarwal, R.; Singh, S.; Pande, J.; Sola,C.; Rastogi, N.; and Seth,P. (2004). Predominant tuberculosis spoligotypes, Delhi, India. Emerg Infect Dis 10(6): 1138-42.
9. Ali, R. M. (2013). Molecular study and Genotyping of Mycobacterium tuberculosis complex isolated in Respiratory Center in Baghdad. Ph. D thesis. Institute of Genetic Engineering and Biotechnology for Postgraduate Studies University of Baghdad.
10. Al- Hajoj, S. (2010). Tuberculosis in Saudi Arabia: Can we change the way we deal with the disease? J. infects. Publ. Heal.53:1-8.
11. Haeili, M.; Darban- Sarokhalil, S.D.; Fooladi, A.A.I.; Javadpour, S.; Hashemi, A.; Siavoshi, F.and Feizabadi, M.M.(2013). Spoligotyping and drug resistance patterns of Mycobacterium tuberculosis isolates from five provinces of Iran. Microbiology Open. 2(6):988-996.
12. Merza, M. A.; Farnia, P.; Salih, A.M.; Masjedi, M.R. and Velayati, A.A.(2010).The most predominant spoligopatterns of Mycobacterium tuberculosis isolates among Iranian, Afghan-Immigrant, Pakistani and Turkish tuberculosis patients: a comparative analysis. Chemotherapy. 56 (3):248-57.
13. Bedrossian, N.; Rahmo, A.; Karam, W. and Hamze, M.(2013). Mycobacterium tuberculosis spoligotypes circulating in the Syrian population: a retrospective study. Int. J. Mycobacteriol. 2:141–147.
14. Gandhi, N.R.; Brust, J. C.M.; Moodley, P.; Weissman, D.; Heo, M.; Ning. Y. Sturm, A.W. and Shah, S. N. (2014). Minimal Diversity of Drug- Resistant Mycobacterium tuberculosis Strains, South Africa. Emerg. Infect. Dis. 20(3): 394–401.
15. Pang, Y.; Zhou, Y.; Zhao, B.; Liu. G.; Jiang, G.; Xia, H.;Song, Y.; Shang, Y.; Wang, S.and Zhao, Y.L.(2012). Spoligotyping and drug resistance analysis ofMycobacterium tuberculosis strains from national survey in China. PLoS One.7 (3):e32976.
16. Merza, M. A.; Farnia, P; Salih, A.M.; Masjedi, M.R. and Velayati, A.A. (2011). First insight into the drug resistance pattern of Mycobacterium tuberculosis in Dohuk, Iraq: Using spoligotyping and MIRU-VNTR to characterize multidrug resistant strains. J. Infect. Public Health.4:41-7.
17. Kisa, O.; Tarhan, G.; Gunal,S.; Albay, A.; Durmaz, R.; Saribas, Z.; Zozio,T.; Alp, A.; Ceyhan, I., and Rastogi, N.(2012). Distribution of spoligotyping defined genotypic lineages among drug-resistant Mucobacterium tuberculosis complex clinical isolates in Ankara, Turkey. Plos one.7 (1):e30331.
18. Feyisa,S.G.; Haeili, M.; Zahednamazi, F.; Mosavari, N.;Taheri, M.M.; Hamzehloo, G.; Zamani, S. and Feizabadi, M. M. (2016). Molecular characterization of Mycobacterium tuberculosis isolates from Tehran, Iran by restriction fragment length polymorphism analysis and spoligotyping. Rev. Soc. Bras. Med. Trop. 49(2): 204–10.
19. Mardassi, H.; Namouchi, A.; Haltiti, R.; Zarrouk, M.; Mhenni, B.; Karboul, A.; et al.(2005). Tuberculosis due to resistant Haarlem strain, Tunisia. Emerg. Infect. Dis. 11:957-61.

20. Al- Hajoj, S.A.; Zozio, T.; Al-Rabiah, F.; Mohammad, V.; Al-Nasser, M. (2007). First insight into the population structure of Mycobacterium tuberculosis in Saudi Arabia. J. Clin. Microbiol.45 (8):2467-73.
21. Zamani, S.; Haeili, M.; Nasiri, M.J.; Imani Fooladi, A.A.; Javadpour, S. and Feizabadi1, M. M. (2016). Research Article Genotyping of Mycobacterium tuberculosis Isolates from Hormozgan Province of Iran Based on 15-Locus MIRU-VNTR and Spoligotyping. Int. J. Bacteriol. 2016: 7146470.

*****