Mycobacterium tuberculosis spoligotypes circulating in the Syrian population: A retrospective study

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ABSTRACT

Objectives: To characterize by spoligotyping clinical isolates of Mycobacterium tuberculosis (MTB) collected between July 2003 and October 2005 from all Syrian provinces (muhafazat).
Methods: All isolates ($n = 96$) were cultured and identified by biochemical characteristics. DNA extracts of these samples were amplified by PCR and genotyped by spoligotyping.
Results: Twelve patterns were identified: 46.8% of the strains belonged to T 1 family; 20.8% to LAM 9; 10.4% to CAS; 9.3% to Haarlem 3; 4.1% to Haarlem 1; 2.1% to Family 34; and 1% to each of Family 36, EAI 5, LAM 1, LAM 8, T 3, and X 3 families. The noticeable absence of the Beijing family was not consistent with the patterns reported in most neighboring countries.
Conclusion: A more inclusive study of the Syrian population is necessary to more accurately identify most of the prevailing families in the country.

Introduction

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. The World Health Organization (WHO) estimates that approximately one-third of the global community is infected with Mycobacterium tuberculosis (MTB) [1]. In 2011, there were an estimated 8.7 million new cases of TB (13% co-infected with HIV) and 1.4 million people died from TB, including almost 1 million deaths among HIV-negative individuals and 430,000 among people who were HIV-positive [1]. Globally, there were an estimated 0.4 million HIV-associated TB deaths in 2011, with approximately equal numbers among men and women. WHO, UNAIDS and the Stop TB Partnership have set a target of halving TB mortality rates among people who are HIV-positive by 2015 compared with 2004 (the year in which TB mortality among HIV-positive people is estimated to have peaked) [2].

Recent studies have shown associations among MTB strains, geographic regions, and human populations. These indicate that specific strains of MTB co-evolved with human subpopulations [3].

Molecular tools have enhanced the understanding of the epidemiology of TB by providing new insight on the transmission, dynamics, source, and spread of MTB [4,5]. Molecular typing methods used to determine clonal and phylogenetic relationships of MTB strains provide useful data about epidemiology of TB in a given population, in a given country, among countries and throughout the world [6]. The spoligotyping method has been widely accepted as a valuable tool for epidemiological studies of TB. It has proven useful for

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the tracking of outbreaks and laboratory cross contamination and for a description of the global spread of TB [7–10].

Many genetic loci within the MTB complex (MTC) genomes are polymorphic and may be used for molecular evolutionary studies [11]. Among these, the Direct Repeat (DR) locus, which consists of alternating identical DRs and variable spacers, can be genotyped using the spoligotyping method [12]. This PCR-based reverse-hybridization blotting technique is easy, robust, cheap, and produces highly diverse portable numerical results [6]. It can be applied directly to clinical samples, thus allowing fingerprinting of a large number of isolates to be performed in a very short time [13].

International databases, such as the SpolDB3.0 have revealed the clonal structure of MTB isolates in different geographical settings. SpolDB4.0 data base further defines super-families specific to certain locations [14]. The abundance of polymorphisms indicates that transposition and homologous recombination are the major events contributing to the diversity of MTB strains [15].

The Syrian Arab Republic is considered an endemic country for TB, with an intermediate burden of disease, an estimated incidence for all cases in 2006 of 32 per 100,000 population and a TB prevalence of 40 per 100,000 population [16].

In the mid-1990s, Syria decided to implement the DOTS (the directly observed treatment, short course) strategy, thus providing the basis to integrate TB treatment into the network of primary health care (PHC) centers. TB control strategy entails the use of short-course regimens of effective drug combinations, direct supervision of treatment for at least the first 2 months, and evaluation of treatment for each patient [17]. The program achieved a cure rate of 92% in Syria [18].

A previous study using double-repetitive element polymerase chain reaction (DRE-PCR) method for the proximity of the repetitive DNA elements IS6110 (a mobile genetic element), PGRS, demonstrates that various genotypes tended to be more common in specific regions than others [19]. The selective distribution of genotype groups, in addition to the observed high level of variability, indicated some level of geographic isolation, and hence separate evolution. It justifies also the initial assignment of the genotypes to major groups according to the observed general amplicon band pattern.

Genotyping MTB in Syria on the national level in parallel with DOTS may prove to be informative and beneficial as DOTS subjects will be closely assessed and monitored for therapeutic response.

This investigation reports on the first nationwide study to characterize clinical isolates of MTB from different regions of Syria. This study could provide some insight into the dynamics of the disease and the possible routes of TB transmission.

Materials and methods

Sampling of patients

Following an agreement with the Syrian Ministry of Health, samples from 96 patients previously treated for pulmonary TB were obtained. These included cases of failure after first treatment, relapse or reinfection. The samples, provided by the Ministry's central laboratory, were collected between July 2003 and October 2005 from all Syrian provinces (muhafazat). They were shipped, accompanied by the related information, to the medical biotech Laboratory of the National Commission for Biotechnology in the Syrian Arab Republic. The research was approved by the responsible ethical committee at the Ministry of Higher Education.
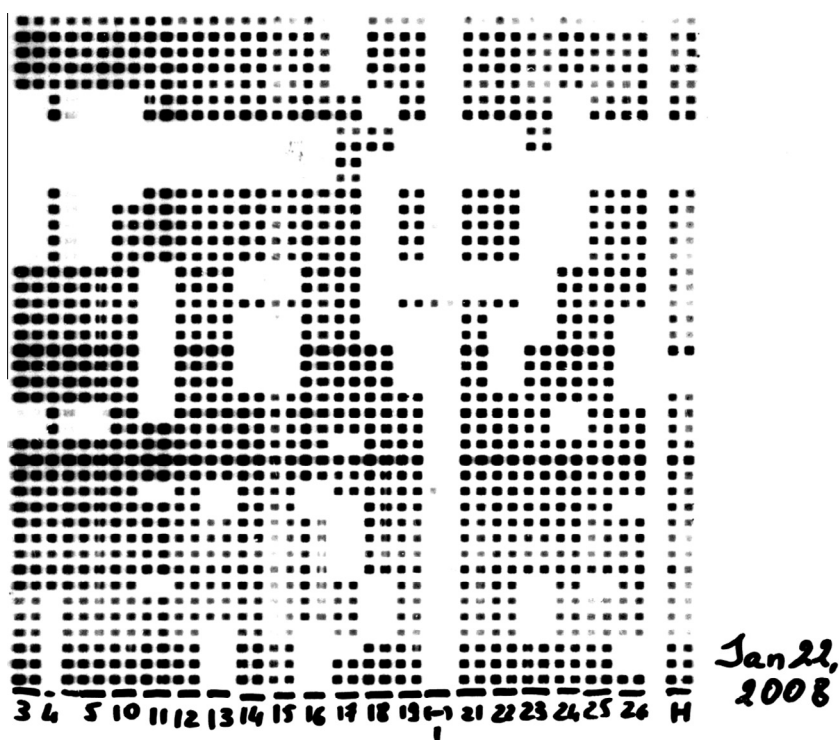

Fig. 1 – An autoradiographic fingerprint prototype of some of the samples tested.

Table 1 – The spoligopatterns of the Syrian samples tested include: the binary formats, the octal formats, the most probable families and the shared types to which they belong (A black square indicates the presence of a spacer, and a blank one its absence).

Table 1: The spoligopatterns of the different samples tested. Included are their binary formats, octal formats & th the most probable families & the shared types (black spaces indicate presence of spacers & blank ones their absence)

Binary Format (spacers 1–43)	Octal Format	Family	ST (shared types)
	7 7 7 7 7 7 7 7 7 7 6 0 7 7 1	T1 (99%)	53
	7 7 7 7 7 7 6 0 0 3 6 0 7 7 1	LAM 9 (98%)	Unclassified
	7 7 7 7 7 4 7 7 0 0 0 1 7 1	Haarlem 3 (48%)	1884
	0 0 7 7 7 7 7 7 7 6 0 7 7 1		Unclassified
	7 7 7 7 7 4 7 7 0 0 0 1 7 1	Haarlem 3 (48%)	1884
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (99%)	41
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	6 7 6 7 7 3 7 7 7 7 7 7 6 0 0	BCG (99%)	
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	7 7 7 7 7 7 7 7 7 4 0 1 7 1	T 1 (99%)	Unclassified
	7 7 3 7 3 6 0 0 0 7 6 0 7 7 1	LAM 9 (97%)	Unclassified
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	7 7 7 7 3 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	367
	4 0 0 0 3 7 7 7 4 1 3 7 7 1	EAI 5 (97%)	8
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	6 7 4 0 7 3 7 7 7 7 7 7 6 0 0	BCG (99%)	685
	7 0 3 7 7 7 4 0 0 0 3 1 7 1	CAS (99%)	25
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 7 7 7 7 7 4 7 7 4 7 6 0 7 7 1	H37Rv	451
	7 7 7 7 7 7 7 4 7 6 0 7 7 1	T 1 (99%)	353
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 0 3 7 7 7 4 0 0 0 3 7 7 1	CAS (99%)	26
	7 7 7 7 7 4 7 7 0 0 0 1 7 1	Haarlem 3 (48%)	1884
	7 7 3 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	926
	4 7 7 6 7 7 0 0 7 7 6 0 7 7 1	LAM 1 (68%)	Unclassified
	4 3 7 7 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	Unclassified
	7 7 7 7 7 7 7 7 7 6 0 7 0 0	T 1 (99%)	51
	7 7 7 7 7 7 7 0 0 0 0 7 7 1	Haarlem 1 (99%)	602
	7 7 7 7 7 7 1 7 7 6 0 7 7 1	T 1 (99%)	771
	7 7 7 7 7 7 7 7 7 6 0 7 3 1	T 1 (99%)	52
	0 3 7 6 3 7 7 7 7 6 0 7 3 1	T 1 (99%)	Unclassified
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 3 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	Unclassified
	0 0 0 0 0 0 0 0 7 7 6 0 7 7 1	Family 36	4
	7 7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 7 7 7 3 7 7 7 7 6 0 7 3 1	T 1 (99%)	52
	7 7 7 7 7 5 7 7 7 6 0 7 7 1	LAM 9 (97%)	120
	7 0 3 7 7 7 4 0 0 0 0 3 1 7 1	CAS (99%)	600
	0 3 7 6 3 7 7 7 7 7 2 0 7 7 1	Haarlem 3 (77%)	Unclassified
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 7 7 7 7 7 6 0 0 3 6 0 7 7 1	LAM 9 (97%)	Unclassified
	7 0 3 7 7 7 4 0 0 0 0 1 7 1	CAS (99%)	22
	7 7 7 7 7 7 7 7 4 0 1 7 1	T 1 (99%)	Unclassified
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	7 7 7 7 7 7 4 7 6 0 7 7 1	T 1 (99%)	Unclassified
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
BLANK			
	7 0 3 7 7 7 6 0 0 0 3 7 7 1	CAS (99%)	1093
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	7 0 3 7 7 6 7 0 0 0 0 0 0 0	Family 34 (99%)	Unclassified
	7 0 0 0 1 7 7 7 7 7 6 0 7 7 1	X 3 (98%)	Unclassified
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 7 7 0 1 7 7 7 7 4 2 0 7 7 1	Haarlem 3 (77%)	1276
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 0 3 7 7 7 4 0 0 0 3 1 7 1	CAS (99%)	25
	7 0 3 7 7 7 4 0 0 0 3 7 7 1	CAS (99%)	26
	7 7 7 7 1 7 7 7 7 6 0 7 7 1	T 1 (99%)	131
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	131
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	7 7 7 7 1 7 7 7 7 6 0 7 7 1	T 1 (99%)	131
	7 7 7 7 3 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	367
	7 7 7 7 7 7 7 0 0 0 0 0 0 0	Family 34 (99%)	46
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 0 3 7 7 7 4 0 0 0 0 7 7 1	CAS (99%)	599
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	0 3 7 6 3 7 7 7 7 6 0 7 7 1	T 1 (99%)	284
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 7 7 7 7 7 7 6 0 0 0 7 7 1	Haarlem 1 (99%)	Unclassified
	0 3 7 0 3 7 7 7 7 6 0 7 7 1	Haarlem 3 (99%)	Unclassified
	7 7 7 7 7 7 7 7 2 0 7 7 1	Haarlem 3 (99%)	50
	7 7 6 3 7 7 7 0 0 0 6 0 7 7 1	LAM 8 (99%)	Unclassified
	7 7 7 3 5 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	1475
	7 7 7 0 0 3 7 7 7 7 6 0 7 7 1	T 3 (99%)	Unclassified
	0 3 7 0 1 7 7 7 7 6 0 7 7 1		Unclassified
	7 0 1 0 4 4 3 4 0 0 0 3 0 7 1	CAS (99%)	Unclassified
	7 7 7 7 7 7 7 0 0 0 0 7 7 1	Haarlem 1 (99%)	602
	0 3 7 0 1 7 7 7 7 6 0 7 7 1	T 1 (99%)	Unclassified
	0 3 7 0 1 7 7 7 7 6 0 7 7 1		Unclassified
	7 0 3 3 5 7 6 0 0 0 0 2 1 7 1	CAS (99%)	Unclassified
	7 7 7 7 7 7 7 0 0 0 0 7 7 1	Haarlem 1 (99%)	602
	7 7 7 7 7 7 4 0 4 7 6 0 7 7 1	LAM 9 (97%)	41
	7 7 7 7 7 7 7 0 0 0 0 0 7 1	Haarlem 3 (99%)	Unclassified
	7 7 7 3 5 7 7 7 7 6 0 7 7 1	T 1 (99%)	1475
	7 7 7 7 7 7 7 7 7 6 0 7 7 1	T 1 (99%)	53
	7 7 7 7 7 7 7 7 4 2 0 7 7 1	Haarlem 3 (77%)	777

Specimen preparation and culture

Processing of sputum specimens was based on liquefaction and decontamination by 2% N-acetyl-L-cysteine-NaOH. Bacterial culture was performed on solid Lowenstein–Jensen medium [20]. Sputum was digested and decontaminated with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria.

Table 2 – Prevalence of the different *Mycobacterium tuberculosis* complex family strains in the Syrian sample pool (*n* = 96).

Family strains	Nb.	Percentage
T1	45	46.8
LAM 9	20	20.8
CAS	10	10.4
Haarlem 3	9	9.3
Haarlem 1	4	4.1
Family 34	2	2.1
Family 36	1	1.0
EAI 5	1	1.0
LAM 1	1	1.0
LAM 8	1	1.0
T 3	1	1.0
X 3	1	1.0

Identification

Identity of strains was based on the following biochemical characteristics: production of Niacin, Nitrate reductase, catalase at laboratory temperature, catalase at 68 °C, hydrolysis of Tween 80 in 10 days, urease in 18 h, and arylsulfatase in 3 days [14].

DNA extraction

A loopful of each culture was suspended in ATL buffer (0.2 mL). DNA samples were extracted in a laboratory free of all mycobacterial products using QIAamp DNA blood Mini Kit (Qiagene). The accompanied procedure was adhered to except for the incubation period that was extended to 3 h. DNA was eluted in 100 µL PCR water (Gibco).

PCR amplification

All DNA samples were positive for the IS6110 as determined by PCR amplification followed by detection by agarose gel electrophoresis using the method of Wilson et al. [21]. PCR set-up was also performed in a remote area, separate from all subsequent procedures. Measures to avoid cross and carry-over PCR contaminations were observed; use of aerosol barrier-fitted pipette tips and of molecular biology-grade water and reagents, in addition to frequent decontamination of surface work areas with diluted bleach and short-UV were regularly performed between experiments.

Spoligotyping was performed for each sample in duplicates and was initially repeated twice to assess the reproducibility of the method. Duplicate positive and negative controls were included in each run. DNA was replaced with water in negative control reactions and DNA of MTB strains H37Rv and Mycobacterium Bovis BCG P3 supplied in the kit were used in positive control reactions. The procedure was performed using a reverse dot-blot spoligotyping kit with chemiluminescent detection (Isogen Bioscience B.V., BT Maarssen, the Netherlands) as follows: 5 µL of mycobacterial DNA was added to a PCR amplification reaction in which the forward DR-primer was biotinylated. Amplification was performed for 25 cycles as recommended by the manufacturer.

Spoligo-membranes were regenerated 3–5 times by washing in 1% SDS at 80 °C for 30 min to 1 h, then in 20 mM EDTA, pH 8.0 for 15 min at room temperature. The membrane was then incubated in ECL detection liquid and re-exposed to a light-sensitive film for a few minutes. Washes were repeated when necessary to eliminate all residual signals. The membrane was then stored at 4 °C until reuse. It is worth noting that the binary to octal translation was done using *spotclust* [22].

Results

A total of 96 clinical samples have been tested by spoligotyping. Fig. 1 shows the autoradiographic fingerprinting of some of the samples tested. In the 43 spacers, black dots reveal a hybridization pattern. Within the same run, each specimen is tested in duplicates and is repeatedly tested in other runs for reproducibility. Each run includes a positive control (H37Rv), BCG, and a blank specimen. These spoligopatterns are then translated into digital binary formats where "1" indicates hybridization and "0" its absence. The digital formats are then expressed as octal formats and the results interpreted as the most probable family to which a particular specimen belongs. Table 1 is a summary of the spoligopatterns of the 96 specimens tested, the most probable families to which they belong and the shared type (ST) designation of each. Table 2 indicates the prevalence of the different MTB complex family strains in the tested samples. Table 3 denotes a detailed analysis of the different MTB complex family clades with the individual shared type (ST) designation, the number of isolates and the corresponding lack of spacers for each.

Discussion

In the present study, among the 96 isolates tested, 12 different spoligotype patterns were identified, indicating the significant heterogeneity of the population studied. However, upon phylogenetic analysis, the vast majority of the genotypes were allocated to only two major clades, namely, the T and LAM 9 clades, which covered 45 (46.8%) and 20 (20.8%) of the MTB strains analyzed, respectively.

According to the SolDB4 database, the "ill-defined" T family is characterized by the absence of spacers 33–36. It clusters various strains whose evolution remains unresolved. This family is widely present in all continents and corresponds to about 30% of all entries in the international database [6]. Most of the T1 family in this study (31.1%) belonged to ST53, 12 (26.6%) to 284; 3 (6.66%) to 131; 2 (4.44%) to 1,475; 2 (4.44%) to 52; and 1 (2.22%) to each of 353, 926, 51 and 771 shared types. Eight (17.77%) of the T1 strains with the following octal descriptions [037017777760771 (*n* = 3), 777777 777740171 (*n* = 1), 437737777760771 (*n* = 1), 037637777 760731 (*n* = 2), 777777774760777 (*n* = 1)] could not be classified under any of the shared types in the SpolDB4 database. One unclassified specimen (1%) with an octal format 777003777760771 belonged to the T3 family (absence of spacers 13–16 and 33–36).

The second predominant family in these results, the LAM 9 family constituted 20.8% (*n* = 20) of the specimen; 13 (65%) belonged to ST 41; 2 (10%) to ST 367; and 1 (5%) to ST 120. Four

Table 3 – Summary of the different clades identified, their shared type designations, the number of isolates and the absence of spacers for the specimen tested.

Clade	Shared types	Number of isolates	Absent spacers/No.
T 1	53	14	33–36/14
	284	12	1–4, 12–13, 33–36/12
	131	3	13–14, 33–36/3
	1475	2	10, 14, 33–36/2
	52	2	33–36, 40/1
			13, 33–36, 40/1
	353	1	26–27, 33–36/1
	926	1	7, 33–36/1
	51	1	33–36, 40–43/1
	771	1	22–23, 33–36/1
	Unclassified	8	1–4, 10–14, 33–36/3
			1–4, 12–13, 33–36, 40/1
			2–4, 13, 33–36/1
			26–27, 33–36/1
			1–6, 33–36/1
			32–38/1
T 3	Unclassified	1	10–16, 33–36/1
LAM 9	41	13	20–24, 26–27, 33–36/13
	367	2	13, 20–24, 26–27, 33–36/2
	120	1	20, 33–36/1
	Unclassified	4	21–28, 33–36/2
			4, 20–24, 26–27, 33–36/1
			4–7, 23–24, 37–38/1
LAM 8	Unclassified	1	9–10, 22–30, 33–36/1
LAM 1	Unclassified	1	2–3, 12, 19–24, 33–36/1
Haarlem 1	602	3	25–36/3
	Unclassified	1	24–36/1
Haarlem 3	1884	3	4–7, 20–35/1
			17–18, 28–38/1
			32–38/1
	1276	1	10–14, 29–31, 33–36/1
	777	1	29–31, 33–36/1
	Unclassified	4	1–4, 12–13, 31, 33–36/1
			1–4, 10–13, 33–36/1
			31, 33–36/1
			28–38/1
CAS	25	2	4–7, 23–34, 37–38/1
	26	2	4–7, 23–34/2
	600	1	4–7, 20–34, 37–38/1
	22	1	4–7, 20–35/1
	1093	1	4–7, 21–34,36–39/1
	599	1	4–7, 20–36/1
	Unclassified	2	4–7, 10, 14, 21–34, 36–38/1
			4–8, 10–12, 14–15, 17–18, 23–34, 37–39 /1
EAI 5	8	1	2–13, 29–32, 34/1
Family 36	4	1	1–24, 33–36/1
Family 34	46	1	25–43/1
	Unclassified	1	4–7, 18, 25–43/1
X 3	Unclassified	1	4–11, 33–36/1

members of this family with the following octal descriptions [777777600360771 (n = 2), 773736000760771 (n = 1) 73777 7404760777 (n = 1)] could not be classified in the database. One specimen (1%) with an octal format 477677 007760771 belonging to LAM 1 family (absence of spacers 3, 21–24, 33–36) and another with an octal format 7763777 00060771 belonging to the LAM 8 family (absence of spacers 22–24, 27–30, 33–36) could not be classified in the database. This Latin American and Mediterranean (LAM) super family is defined by the simultaneous absence of spacers 21–24, and 33–36 [14]. In general, the LAM family is found to be the highest in Venezuela (65%),

in the Mediterranean basin (e.g., 34% in Algeria, 55% in Morocco, 30% in Spain), and in the Caribbean region (30% in Cuba and Haiti, 17.4% in French Guiana, 15% in French Caribbean islands [23]. Syria's geographical location in the Mediterranean basin and the great tendency of the Syrians, since ancient times, to immigrate to Venezuela, Central America and to some other African countries, with intermittent visits to their mother country could explain these findings.

The Central Asian (CAS) lineage covered 10.4% (n = 10) of the samples tested; 2 (20%) belonged to ST 25; 2 (20%) to ST 26; and 1 (10%) to each of 600, 22, 1093 and 599 STs. CAS members with

the following octal descriptions [701044340003071 (*n* = 1) and 703357600002171 (*n* = 1)] could not be classified in the database. This family is characterized by the absence of spacers 4–7, and 23–34. Strains of this family have been reported in different countries of the Middle East (Iran, Pakistan, India and Afghanistan) [24,25] and to a lesser extent in other regions (Africa 5.3%, Central America 0.1%, Europe 3.3%, Far-East Asia 0.4%, North America 3.3%, Oceania 4.8%). Nowadays, the CAS 1-Delhi family is essentially localized in the Middle-East and Central Asia, more specifically in South-Asia (21.2%) and preferentially in India (75%) [25,26]. Interestingly enough, recently this family composed 14.2% of the Iranian and Afghan MDR (multidrug resistant) TB patient communities [25].

In the Haarlem (H) lineage, 9.3% of the samples belonged to Haarlem 3 and 4.1% to Haarlem 1 families. Of the Haarlem 3 family, 3 specimens (33.3%) belonged to ST 1884, and 1 (11.1%) to each of ST 1276, 50 and 777. Three strains (33.3%) with the following octal descriptions [037637777720771 (*n* = 1), 037037777760771 (*n* = 1) and 777777777000171 (*n* = 1)] could not be classified. Concerning the Haarlem 1 family, 3 (75%) belonged to ST 602 and 1 (25%) with the octal format 777777760000771 could not be classified. Haarlem is characterized by the absence of spacers 26–31 and 33–36. In Europe, this lineage represents about 25% of the isolates [7]. Outside Europe, the Haarlem strains were mainly found in Central America and the Caribbean (about 25%), suggesting a link of Haarlem to the post-Columbus European colonization [24]. Today its widespread distribution in different geographical regions of the world such as Asia, Europe and Africa has been documented [27]. However, these organisms of European descent were rarely found in India [28]. This family accounts for more than half (57.1%) of all clustered strains among Iranian MDR-TB patients [25], thus, necessitating from an epidemiological point, extensive surveillance of MDR strains because they might cause serious outbreaks. It is characterized by the absence of spacers 29–31, and 33–36 (prototypes ST 127 and/or ST 777). The ST 777 pattern isolated in this specimen is mainly found in Saudi Arabia, Kazakhstan, Russia and Georgia [29]. Thus, the presence of Haarlem is related to the historical contacts that existed between Syria and most of the European communities, in addition to Arab countries such as Saudi Arabia where many Syrians went seeking work.

In this study, 2 (2.1%) specimens belonged to family 34 (absence of spacers 25–43), one (50%) with an ST 46 designation and another one (50%) with an octal format 70377677000 0000 was unclassified; 1% (*n* = 1) of the samples belonged to family 36 (ST 4), characterized by the absence of spacers 1–24 and 33–36. Until the present, this family is solely of USA origin [22].

Nearly 1% (*n* = 1) of the samples with a database signature ST 8 belonged to the East-African-Indian (EAI 5) lineage. The EAI super family is characterized by the presence of spacer 33 and the absence of spacers 29–32 and 34. It is highly prevalent in South-East-Asia, particularly in the Philippines (73%) [30], Myanmar and Malaysia (53%) [31], and in 17% of MTB isolates from patients with pulmonary tuberculosis in Mumbai, India [29]. In addition, it is one of the most frequent strains (12.9%) in Iranian and Afghan MDR-TB patient communities [25].

Nearly 1% of the samples belonging to the X3 family and with an octal format 700177777760771 could not be classified. The X super family is defined by the simultaneous absence of spacers 4–12, 18 and 33–36. It is subdivided into at least three distinct families: X1 to X3 [26]. Nowadays, this group of strains is currently correlated with African-Americans [30].

Noticeably enough, in the samples tested, none belonged to the Beijing family (characterized by the absence of spacers 1–34), which is quite prevalent in the countries of the Middle East and Far East. Actually, they represent around 50% of the strains in Far-East-Asia, 16.5% in the Middle East and Central Asia, 17.2% in Oceania [5], 10% in India [28], and 13% of isolates globally [5]. In addition, it accounts for 15.5% of the isolates in the MDR – TB in Iranian and Afghan patients [25]. **A** previous study run in Syria [32] indicated the absence of any Beijing strain in the samples tested. Such absence should be further confirmed through larger sampling pools given the proven presence of these strains in neighboring countries (Turkey, Palestine, Saudi Arabia) [33]. On the other hand, it is possible that the Syrian population is less prone to infection by the Beijing family of strains [34]. This has been earlier hypothesized for other cases of limited spread of the Beijing genotype [35].

Conclusion

The results of this study fit fairly well with the molecular epidemiology of MTB genotypes described in the international database. Several unclassified strains were detected, 8 belonging to the T 1 family, 4 to each of LAM 9 and Haarlem 3, 2 to CAS, and 1 to each of T 3, LAM 8, LAM 1, Haarlem 1, EAI 5, Family 34 and X 3 families. This indicates a great variability and polymorphism in the strains studied. However, the absence of Beijing is not consistent with the patterns reported in neighboring countries, despite the fact that Syria is open to workers from the Far and Middle East (Philippines, Bangladesh, Nepal, Srilanka, India, Pakistan, Iran, Russia, Iraq, etc.), and from Africa (Egypt, Sudan, Ethiopia, etc.). A more inclusive study of the Syrian population is necessary to more accurately identify most of the prevailing families in this country and to further extrapolate the benefits for prevention and possibly treatment of tuberculosis.

Conflict of interest

None declared.

Acknowledgments

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