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Original Article

Spoligotyping of *Mycobacterium tuberculosis* isolates using Luminex®-based method in Lebanon

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Abstract

Introduction: Data about the genotypes of circulating *Mycobacterium tuberculosis* isolates (MTB) in Lebanon are scarce. This study was undertaken to reveal the spoligotypes of MTB isolates recovered from patients in Lebanon.

Methodology: MTB isolates from 49 patients living in Lebanon were recovered and identified. The samples were heat killed and subjected to DNA extraction. Spoligotyping was performed using microbeads from TB-SPOL Kit and the fluorescence intensity was measured using Luminex 200®. Generated patterns were assigned to families using the SITVIT2 international database of the Pasteur Institute of Guadeloupe and compared.

Results: The spoligotyping of the 49 MTB isolates revealed that 31 isolates belonged to Lineage 4 (Euro-American, 63.3%), 12 to Lineage 3 (East- African Indian, 24.5%), 3 to Lineage 2 (East Asian, 6%) and 2 were unknown. Over half of the genotypes (16 of 30) harbored SIT127 supposed to belong to the L4.5 sublineage. One isolate belonging to the rare Manu-Ancestor SIT523 was recovered for the first time in Lebanon, being associated with highly virulent extensively drug-resistant (XDR) MTB phenotype.

Conclusion: The application of the Spoligotyping Multiplex Luminex® method is an efficient, discriminatory and rapid method to use for first-lane genotyping of MTB isolates. Though humble numbers were tested, this study is one of the first to describe the genomic diversity and epidemiology of MTB isolates of Lebanon, and suggests an increasing prevalence of SIT127 in the country.

Key words: Spoligotyping; Luminex; *Mycobacterium tuberculosis*; Lebanon.

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Introduction

Mycobacterium tuberculosis (MTB) remains a major health problem globally including Lebanon [1-3]. Genotyping of MTB and determination of local phylogenetic lineages are becoming crucial for Tuberculosis (TB) outbreaks surveillance, transmission patterns deciphering, and infection control strategies [4].

Different molecular tools have been proposed for the molecular genotyping of MTB, where the IS*6110* method has been considered the "gold standard" since 1993 [5]. However, this method is an expensive, laborious and lengthy methodology. It requires specific software to analyze the restriction fragment length polymorphism (RFLP) band patterns, all of which make the interpretation and exchange of the data difficult [6]. On the other hand, spoligotyping was the second most widely used method for MTB complex genotype after IS*6110*-based fingerprinting and gained increased international acceptance as a rapid, first line and discriminatory test [7-10]. It relies upon polymerase chain reaction (PCR) amplification of a single direct repeat (DR) locus which consists of alternating identical DRs and variable spacers harboring 36 base pairs (bp) direct repeats interspersed with unique 34–41 bp spacer sequences. The entire DR locus is amplified by PCR, using two inversely oriented primers complementary to the sequence of short DRs. [7]. The PCR products are hybridized to a membrane containing 43 oligonucleotides corresponding to the spacers from MTB $H_{37}Rv$ and *M.bovis* BCG. The presence or absence of each of those 43 spacers in the DR region of

the analyzed isolate will be represented as the pattern of positive or negative hybridization signals [7]. The hybridization signals are detected by chemiluminescence through biotin labeling of the PCR products (one of the primers is biotinylated) and a streptavidin-peroxidase conjugate system that could be visualized by either autoradiography or using the Luminex® technology [11,12]. In this latter, the synthetic spacer oligonucleotide probes are immobilized on microspheres by means of covalent coupling. The detection is then achieved via fluorochromes attached to the beads and to the hybridized PCR product. The Luminex® platform provides greater robustness and reproducibility since it eliminates the membrane steps with its subjective visual data interpretation, replaced by mathematical cut-offs [11,12].

International databases, such as the World Spoligotyping Database, SpolDB4.0 and SITVIT2, have revealed the clonal structure of MTB isolates in different geographical settings [13,14]. The SITVIT2 updated database includes classification for spoligotypes and description of the genetic families of MTB for 111,635 clinical isolates from 169 countries of patient origin (131 countries of isolation, representing 1032 cities); these isolates contain more than 7,100 patterns of spoligotyping that are grouped into 3882 SIT (Shared International Type) codes [14]. The evolution of DR, renamed CRISPR (Clustered-Regularly-Interspersed-Short-Palindromic-Repeats) has enabled the analysis of MTB population structure, and the classification of MTB in seven lineages based on Whole-Genome-Sequencing (WGS) was shown to be congruent to spoligotyping-based classification in most cases [15]. The WGS-based approach revealed major lineages, designated as L1 to L7 [16]. Some lineages are made-up of many sublineages; for L4, some known sublineages are found in Africa, e.g. Uganda (4.6.1), Cameroon (4.6.2), South Africa (4.4), in Asia (L2/Beijing and L3/Central Asia), in America and Europe: Latin American-Mediterranean/LAM [4.3], X [4.1.1], Ghana [4.1] and Haarlem [4.1.2]. L1 is found in South-East Asia and East Africa. The L1-L4 lineages affects humans, whereas other lineages (L5-L6) such as the *M. africanum*, *M. bovis*, *M. caprae* and *M. microti* families primarily affect animals [17].

Several reports addressed the genotyping profiles of MTB isolates in different parts of the world, however, this characterization is scarce in Lebanon [18,19]. Hence, the goal of this study was to reveal the genotyping diversity of MTB complex strains in Lebanon. Spoligotyping using a Luminex® assay was used and the generated data were compared to those published with known patterns especially for the Mediterranean and North-East African regions.

Methodology

A total of 49 clinical MTB isolates were recovered from the sputum of both Lebanese (n = 38) and non-Lebanese (n = 11) patients who were suspected of having tuberculosis during the period of 2010-2012. Isolates were sent with unique consecutive identification code to ensure no link to patients. Thus, Institutional Review Board (IRB) approval or patient consents were not required for this study. The MTB isolates were recovered at the Clinical Microbiology Laboratory of the Department of Pathology and Laboratory Medicine at the American university of Beirut Medical Center (AUBMC). This accredited laboratory by the College of American Pathologists (CAP) acted as the reference TB laboratory to the Lebanese Ministry of Public Health from 1994 till February 2017.

Culture and identification of mycobacterial isolates

Sputum specimens were digested-decontaminated according to standard procedure, as reported earlier [2,3,19]. Briefly, the processed specimens were inoculated into both a Middlebrook 7H9 broth (Mycobacterial Growth Indicator Tube- MGIT, Merck, Darmstadt, Germany) and a solid-based medium (Löwenstein-Jensen, Merck, Darmstadt, Germany). The recovered isolates were identified using MGIT-TBc identification test (Becton Dickinson, Sparks, USA) which differentiates MTB from Non-MTB. The quality control of testing was ensured using the reference MTB strain (H37Rv, ATCC 27294).

DNA extraction and spoligotyping

Inactivated strains were forwarded to Center for Infectious Disease Research (CIDR) for DNA extraction and Spoligotyping procedure. The DNA was extracted using QIAamp® DNA mini kit (Qiagen, Hilden, Germany) following the manufacturers' instructions [20]. Spoligotyping was carried out using a spoligotyping kit (TB-SPOL, Beamedex®, Orsay, France) that contains all materials needed except the *Taq* Polymerase. PCR product was produced using biotinylated primers DRa (GGTTTTGGGTCTGACGAC) and DRb (CCGAGAGGGGACGGAAAC) 10 µM stock, dNTPs (2 mM, stock), MgCl2 (25 mM, sotck), buffer flexi (5X), sterile water and HotStart Taq DNA Polymerase (units) (Qiagen, Hilden, Germany) using a published

protocol [12]. PCR conditions were 3 min at 95°C, followed by 24 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. Negative control contained no DNA, and positive control contained either *M. bovis* DNA or *MTB* strain H37Rv DNA provided within the kit. Only 5 µL of each biotinylated PCR-amplified DNA were applied to freshly prepared microbeads working mix in 96-well flat-bottom plate (Corning Incorporated, NY, USA) according to the multiplexed direct hybridization protocol provided in the supplier work guide (Beamedex®, Orsay, France). Fluorescence produced from Streptavidin-Phycoerythrin binding in samples and control wells is then measured, according to the presence or absence of each of the 43 groups of beads on the flow cytometry-based Luminex 200® system (Bio-Plex, Bio-Rad, Hercules, USA). Median Fluorescence Intensity (MFI) values were obtained via Bio-Plex Manager Software 6.1 version. Raw csv files were converted with Excel spreadsheet files into .xls format (Microsoft, Redmond, WA, USA) in order to determine positive spacers in each sample and control. Cut-off values determining positive ("n", truetype monotype sort font 9 or 10 to get ultrametric full square patterns) and negative spacers ("o", truetype monotype sort font 9 or 10 to get ultrametric empty square patterns) were calculated as mean of signal from three blank wells.

Definition of lineage and strains genotyping

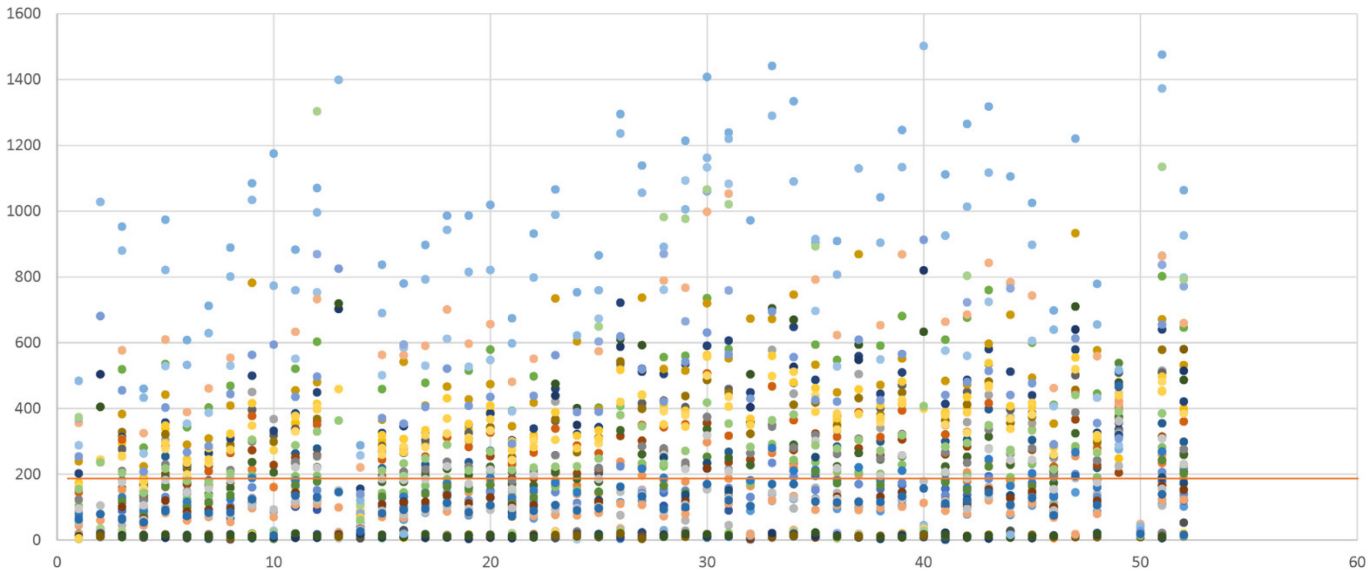
For identification of genetic families and lineages, the 43-digit binary spoligotyping codes (n/o) were individually entered into the SITVIT2 database of Pasteur Institute of Guadeloupe, and compared with existing spoligotyping-based taxonomical standards [14]. Codes that do not provide lineage information are referred to as 'orphan' when unique and 'new' whereas codes in cluster were referred as 'NEW'. Based on the regions of difference (RD) and WGS classification systems using SNPs and large sequence polymorphisms (LSP) and correlation to spoligotyping signatures, the seven major global MTB complex lineages are defined; L1 is Indo-Oceanic/named East-african Indian or EAI in spoligotyping-based nomenclature), L2 is East-Asian (and includes Beijing sublineages), L3 is East-African-Indian/also named CAS or Central Asian in spoligotyping-based nomenclature, L4 is the Euro-American, historically designated as "T" with at least 10 sublineages, L5 is *M. africanum* West African I, L6 is *M. africanum* West African II and L7 is the Ethiopian-specific lineage.

Results

Genotypic lineage identification

The 49 MTB isolates were identified for the presence and the absence of 43 spacers, and characterized in both binary and octal spoligotypes. Our threshold values were calculated as mean of three blank assays for each genetic marker (spacer). In this list each

Figure 1. Semi-log curve showing MFI values distribution for 43 genetic markers targeted in 49 MTB clinical isolates*.



*The threshold of MFI for each spacer is calculated as mean of signal from three blank wells. Red line indicates threshold of MFI for the 43 spacers (200 U.F) as proposed by the TB-SPOL® manufacturer. (X: spacer number, Y: MFI (Median Fluorescence Intensity). *Fluorescence raw mean value of each sample was present in a different color to enhance visibility.*

spacer is defined by Median Fluorescence Intensity (MFI) of its related cut-off value: Sp1: 25, Sp2:25, Sp3:29, Sp4:23, Sp5:20, Sp6:37, Sp7:20, Sp8:20, Sp9:17, Sp10:21, Sp11:20, Sp12:23, Sp13:46, Sp14: 21, Sp15:20, Sp16:24, Sp17:25, Sp18:22, Sp19:23, Sp20:33, Sp21:27, Sp22:21 Sp23:23, Sp24:18, Sp25:42, Sp26:37, Sp27:30, Sp28:34, Sp29:24, Sp30:36, Sp31:24, Sp32:20, Sp33:31, Sp34:22, Sp35:23, Sp36:21,Sp37:18, Sp38:20, Sp39:18, Sp40:22, Sp41:16, Sp42:23, Sp43:18 (Figure 1).

The most represented lineages were 63.3% for Lineage 4 (Euro-American-T), 24.5% for Lineage 3 (CAS), and 6% for Lineage 2 (East-Asian). Lineages 1 (EAI) 5 and 6 (*M. africanum and M. bovis*) and L7 were not detected. 16 isolates out of 31 belonging to L4 are likely to belong to the L4.5 sublineage, here designated as SIT127. One isolate, a rare SIT523 type, was revealed to be associated with an highly virulent XDR MTB phenotype (Table 1).

Discussion

This study from Lebanon revealed a high diversity of strains among the analyzed MTB isolates, where the Lineage 4 predominates, in particular "NEW-1" (L4.5), Haarlem (L4.1.2), CAS and CAS1-Delhi, (L3), and where other Euro-American (L4) and a SIT523 were also present. In brief, the most predominant shared types were SIT127 ("NEW1", 32.6%), SIT25 (CAS1, 4%) and SIT26 (CAS1-Delhi, 6%). Compared to a recent study from Lebanon, which characterized 13 MTB isolates by WGS, our findings showed close rates in the prevalence of lineage 4 (63% vs 53.8%) and lineage 2 (6% vs 7.7%), while higher rates of lineage 3 (24.5% vs 7.7%). However, no lineage 1 or *M. bovis* were detected among our isolates as was the case in the mentioned study (L1 and *M. bovis,* each at 15.4%) [19].

Phylogenetically, the L4.5/SIT127 sublineage was recently suggested to have spread from China to Iran around 1000 to 800 years ago through Xinjiang/Uyghur from an ancestral clone present in China, possibly via expansion of the Mongol Yuan empire [21,22]. Mokrousov *et al*. showed a correlation of their results with migration data, that suggests an East to West slow but definitive migration of lineage 4.5, from China to Iran and Afghanistan and Pakistan during the last 40 years, in relation to Multi-drug-resistance emergence and spreading [21,22]. Indeed, if this lineage was considered in the past minor and underestimated, new analysis of its clones show high level of multidrug resistance, a phenomenon that we did not yet observe in our study in Lebanon. The SIT127 transmission from the Far East to Middle East could be explained historically by many theories like the Silk Road and ancient commercial travels. However recent dramatical events such as the Afghanistan war (1979), the Iran-Iraq war (1980-1988), the first and second Iraq war (1990-1991 and 2003-2011) and finally the Syrian war and the Syrian refugee's population movement that followed (2011-2018), all these events created dramatic changes on population flows and on the tuberculosis outbreak in this region of the world [23,24]. This possibly also explains the high prevalence of SIT127 in neighboring countries at the same period (Iran 17% overall; 26% in Southwestern part), Iraq (3%), and Saudi Arabia (1.5%) [25-27]. ST127 isolates are found in many countries with 13 different national origins in SITVIT2 database (Afghanistan n = 8, China n = 10, UK n = 1, India n = 6, Iran n = 42, Iraq n = 12, Italy n = 1, Kirghizstan n = 4, Lebanon n = 1, Oman n = 1, Pakistan n = 4, Romania n = 1, and Saudi Arabia n = 7) (http://www.pasteur-guadeloupe.fr:8081/SITVIT2/index.jsp). Moreover, more than 60% of ST127 isolates are reported from Armenia, Austria, Finland, Georgia, and Russia [10].

L3, the Central-Asian lineage, appears to have a moderate prevalence rate among MTB isolates in our study. This lineage is mainly found in Central Asia, preferentially North India and Pakistan, some countries of the West Asia (Afghanistan), and several countries in East and North-East Africa such as Sudan, Tanzania, Ethiopia [28-30]. In our study, the CAS1-Delhi sublineage of the L3 totaled 10.2% of the tested isolates. Generally, the CAS sublineages displayed more diverse spoligotypes, SIT25 and SIT26 being the most predominant ones, which might be linked to a yet undeciphered evolutionary dynamics of the L3 within the MTB isolates [31]. A high prevalence of CAS1-Delhi is indeed observed in Sudan (53.9%, SIT25 (35%) [32], Iraq (41.8%; SIT25 (14.2%), SIT26 (6%)) [27], Syria (10.4%; SIT25 and SIT26 (20%)) [33], and Pakistan (CAS 39%; SIT26 39%) [34].

The L4 genotype SIT53 by its ubiquitous characteristics, is definitively associated to more than one sublineage although it is also encompassed in L4.1.1 Ghana [16]. It showed a low prevalence (2%) in our study compared to earlier study from Lebanon (20%) [18], as well as to those reported from Egypt (33.8%) [35], Syria (31.3%) [33], Iraq (26.8%) [27], Iran (6.1%) [25], Algeria (25.3%) [36], Tunisia (10.5%) [37], and Italy (11.7%) [38]. This difference in prevalence between our results and others could be attributed to the low number of MTB isolates screened in this study.

Table 1. Spoligotyping profiles among the 49 MTB clinical isolates from patients residing in Lebanon *. from left to right: Identification number, binary spoligotype, octal spoligotype, lineage (SOL) / L1-L7 sublineage (SL) and SIT (spoligotyping international type as found in SITVIT). An interrogation mark shows that lineage assignation is not certain but only likely (some false hybridization spots, although rare remains possible).

Identification Number	Binary Spoligotype	SL	SIT
Lebanon-1	vvvvooooovvooovvvvvvvvvvvvvvvvvvvooooovvvovvv	740617777760731	L4/new, close to SIT2075
Lebanon-2	ooooooooooovooooooooooovooooovooooovvvvvvvvv	000200040203771	L2/SIT1?
Lebanon-3	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvvv	577777777420771	L4.5/SIT127
Lebanon-4	voovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvvv	477777777420771	L4/Orphan, L4.5?
Lebanon-5	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvvv	577777777420771	L4.5/SIT127
Lebanon-6	voovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvvv	477777777420771	L4/Orphan, L4.5?
Lebanon-7	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvvv	577777777420771	L4.5/SIT127
Lebanon-8	voovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvvv	477777777420771	L4/Orphan, L4.5?
Lebanon-10	vvvooooovvvvvvvvvvvvvvvooooooooooooooovvvvvvv	703777740000771	L3/SIT357
Lebanon-11	vvvooooovvvvvvooooooooooooooooooooooovvvvvoo	703740000000760	L3/SIT864
Lebanon-12	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-13	vvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvovooooovvvvvv	777777777720771	L4/SIT50 L4.5 or L4.1.2
Lebanon-14	ooooooooooooooooooooooooooooooooooovvvvvvvvv	000000000003771	L2/SIT1
Lebanon-15	ovvoovvvvvvvvvovvvvvvvvoovvvvvvvvvvooovvvvvvv	317767747761771	U/new
Lebanon-16	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-17	vvvooooovvvvvvvvvvvvvvvvoovvvvvvvvvvooooovvvvvv	703777747760771	L3/new
Lebanon-18	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-19	ooooooooooovvvvvvvvvvvvvvvvvvooovooooovvvvvv	000177777420771	L4.5?/new
Lebanon-20	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-21	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-22	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-23	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-24	vvvooooovvvvvvvvvvvvvvvooooooooooooovvvvvvvvv	703777740003771	L3/SIT26 (CAS-Delhi)
Lebanon-25	vvvooooovvvvvvvvvvvvvovooooooooooooovvvvvvvvv	703777640003771	L3/SIT2145
Lebanon-26	oooovvvvvvvvvvvvvvvoovvvvvvvvvvvvvvvooooovvvvvv	037776377760771	L4/NEW-CLUSTER1
Lebanon-27	vvvooovvvvvvvvvvvvvooooooooooovooooovvvvvvvvv	707777000203771	L3?/new
Lebanon-28	vvvooooovvvvvvvvvvvvvvvooooooooooooovvvvvvvvv	703777740003771	L3/SIT26 (CAS-Delhi)
Lebanon-29	oooovvvvvvvvoovvvvvvvvvvvvvvvvvvvvooooovvvvvv	037637777740771	L4/new close to SIT284 (Δ32)
Lebanon-30	oooovvvvvvvvvvvvvvvoovvvvvvvvvvvvvvvooooovvvvvv	037776377760771	L4/NEW-CLUSTER1
Lebanon-31	oooovvvvvvvvvvvvvvvoovvvvvvvvvvvvvvvooooovvvvvv	037776377760771	L4/NEW-CLUSTER1
Lebanon-32	oooovvvvvvvvvvvvvvvoovvvvvvvvvvvvvvvooooovvvvvv	037776377760771	L4/NEW-CLUSTER1
Lebanon-33	vvvooooovvvvvvvvvvvvvvvooooooooooooovvoovvvvv	703777740003171	L3/SIT25
Lebanon-34	vvvooooooovvvvvvvvvvvvvooooooooooooovvvvvvvvv	700377740001771	L3/SIT1151
Lebanon-35	vvvooooovvvvvvvvvvvvvvvooooooooooooovvvvvvvvv	703777740003771	L3/SIT26 (CAS-Delhi)
Lebanon-36	oooovvvvvvvoovvvvvvvvvvvvvvvvvvvvooooovvvvvv	037637777741771	L4/SIT284
Lebanon-37	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-38	vvvooooovovvvovvvvvvvvvooooooooooovvvvvvvvv	702737740003771	L3/new, close to SIT1092
Lebanon-39	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-40	vovovvvvvvvvvvvvvvvvvvvvvvvvvvvooovvvooovooooovvvvvv	537777763420771	L4.5?/new
Lebanon-41	ooooovoovvvooooooooooooooooooooooovvvvvvvvv	004600000003771	L2/SIT1?
Lebanon-42	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-43	vvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvooooovvvvvv	777777777760771	L4/SIT53 L4.1.3?
Lebanon-44	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-45	vvvooooovvvvvvvvvvvvvvvoovvvvvvvvvooooovvvvvv	703777747760371	L3?/
Lebanon-46	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-47	vovvvvvvvvvvvvvvvvvvvvvvvvvvvvvooovooooovvvvvv	577777777420771	L4.5/SIT127
Lebanon-48	vvvooooovvvvvvvvvvvvvvvooooooooooooovvoovvvvv	703777740003171	L3/SIT25
Lebanon-49	vovovvvvvvvvvvvvvvvvvvovvvvvvooovooooovvvvvv	537777757420771	L4/new L4.5?
Lebanon-50	vvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvvv	777777777777771	SIT523

The Manu ancestor (SIT523) is the type harboring all 43 spacers and represent both an ancestral type but also in some cases an artefact due to co-infection by two strains belonging to two different lineages [39]. It was unexpectedly detected in one MTB isolate in our study. Such strains were previously reported in high proportion in Egypt [35] and Sudan [28]. The isolation of a remarkable SIT523 XDR-TB strain points to further WGS-based investigation of this isolate.

Interestingly, the high prevalence of orphan MTB genotypes (32.6%) detected in our study could be attributed to the use of spoligotyping as a sole technique, comparable to what was reported in other studies [30,40]. This points out the need for coupling spoligotyping to other techniques, such as Variable Number of Tandem Repeat (VNTR) analysis and WGS to improve taxonomical assignments and possibly to reveal antituberculosis drug resistance profile.

Conclusion

This study provides a first insight about the genotypes of *M. tuberculosis* isolates circulating in Lebanon using the spoligotyping Luminex® based-method. Such findings constitute a baseline for future comparison in evolutionary genotypic trends among MTB isolates in this country.

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