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First insight into the genetic population structure of *Mycobacterium tuberculosis* isolated from pulmonary tuberculosis patients in Egypt

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ABSTRACT

The present study aimed to assess the population structure of *Mycobacterium tuberculosis* (MTB) isolates from Egypt. A total of 230 MTB isolates were analysed using spoligotyping, large sequence polymorphism (LSPs), mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) typing and multi-locus sequence typing (MLST). The majority of isolates (93.0%) belonged to lineage 4, including 44.3, 13.4 and 10.8% of the ill-defined T clade, LAM and Haarlem families, respectively, and lineage 3 was identified in 7.0 % of the isolates. MIRU-VNTRs typing allowed efficient discrimination of the spoligotype-defined clusters, including spoligo-international types (SIT) 53, 34, and 4, into 56 patterns, including 13 clusters and 43 unique patterns. A new SNP at position 311614 was identified in all six isolates to form the biggest MIRU-VNTR cluster, which suggested a recent clonal expansion. This SNP could possibly be used as a genetic marker for robust discriminations of Egyptian MTB isolates belonging to SIT53. The combination of spoligotyping, 12 MIRU-VNTRs loci and MLST provided insight into the genetic diversity and transmission dynamics of the Egyptian MTB genotypes and could be a key to implementation of effective control measures by public health authorities.

Keywords: Genotyping, MTB Lineages, Egypt.

1. Introduction

Tuberculosis (TB) remains a major global health problem, causing 9 million new cases and 1.5 million deaths in 2013. Most of TB cases are reported from Asia (56%) and Africa (29%), whilst only a small number of cases (8%) occurred in the Eastern Mediterranean Region (EMR) [1]. Egypt is a transcontinental, economically diversified middle-income country with 82 million inhabitants. It is bordered by the Mediterranean Sea to the north, and categorized as an EMR member of the World Health Organization. In 2013, Egypt ranked amongst mid-level TB-burdened countries, with reported prevalence and incidence rates (includes HIV+TB) of 27 and 16 per 100,000 inhabitants, respectively [1].

Over the last decades, many genotyping tools such as spoligotyping [2], mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) typing [3] and multilocus sequence typing (MLST) [4,5] have provided insights into the genetic diversity, transmission dynamics and phylogenetic analysis of the *Mycobacterium tuberculosis* complex (MTBC) lineages, including biomedical and epidemiological characteristics specific to each phylogenetic lineage strain [6-8].

The MTBC associated to human infections consists of 7 main lineages, each defined by lineage-specific markers. Lineage 3 includes the spoligotype-defined Central Asian (CAS) family, which belongs to PGG1 and spreads mainly across North India and East Africa. Lineage 4 is characterized by the deletion of spacers 33–36 in the direct repeat (DR) locus and classified into 5 clades (T, Haarlem, LAM, S and X). It is a modern phylogenetic group belonging to the principle genetic group 2-3 (PGG2-3) and is the prevalent lineage in Europe and the Americas, although it has spread across different regions of Africa and the Middle East [9-11].

The few investigations that have addressed the genetic diversity of MTBC lineages in Egypt have indicated the predominance of lineage 4 amongst Egyptian isolates [12-14]. Nonetheless, the epidemiology and molecular characterisation of MTBC strains in Egypt remain largely unknown. The current study aimed to use molecular genotyping tools such as spoligotyping, MIRU-VNTR

67 and MLST typing for elucidating the genetic diversity and characterising the prevalent lineages of
68 *Mycobacterium tuberculosis* (MTB) strains infecting Egyptian patients. It also aimed to contribute
69 better understanding of the population structure, phylogenetic relations and the transmission
70 dynamics of MTB strains in Egypt and surrounding EMR countries.

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2. Material and Methods

73 2.1. Samples collection

74 During the period 2012-2014, MTB isolates from Egyptian patients were processed and cultured
75 either on site or at intermediate laboratories and sent to the TB Supranational Reference Laboratory
76 (TB-SNRL), Central Public Health Laboratories, Ministry of Health and Population, Egypt. From
77 this MTB isolate pool, 230 were randomly selected for the present study. In addition, patients'
78 epidemiological and demographic data were obtained from medical records at TB-SNRL (Table 1).
79 These patients were from 16 Egyptian governorates, namely, Cairo, Alexandria, El Giza, El
80 Fayoum, El Dakahlia, El Gharbia, El Minya, Suez, Kafr elsheik, Damietta, El Behira, Helwan, El
81 Sharqia, El Ismailia , Qena and North Sinai (Figure 1).

82 2.2. Sample processing and MTBC identification

83 Sputum samples were decontaminated and cultured on Löwenstein–Jensen medium (LJ). The
84 clinical isolates were identified as members of the MTBC by biochemical and cultural
85 characteristics. DNA was extracted by the boiling method. Afterwards, EDTA was added at the final
86 concentration of 1 mM, and kept at -20 °C until used for further molecular analysis.

87 To confirm the species as a member of the MTBC, *rpoB* gene sequencing and comparison with the
88 type strain (H37Rv) sequence were performed according to the procedure described by Poudel *et al.*
89 (2012) [15].

90 2.3. Spoligotyping

91 All MTB isolates were analysed by spoligotyping, as reported by Kamerbeek *et al* (1997) [2].
92 Briefly, a PCR-based reverse-hybridisation technique was used in which the DR region was

93 amplified with a primer pair. The PCR products were hybridized to a set of 43 oligonucleotide
94 probes corresponding to each spacer and covalently bound to the membranes.

95 **2.4. LSPs**

96 Isolates with orphan spoligotype patterns were identified with a PCR-based method using specific
97 primers for the expected Regions of Difference (RD) for each lineage, as carried out by Gagneux *et*
98 *al* (2006) [9].

99 **2.5. MIRU-VNTR typing**

100 MIRU-VNTR typing was conducted according to Supply *et al* (2006) [3], and the number of
101 repeats for each locus was determined. MIRU-VNTR loci, namely, MIRU (10, 16, 26 and 40),
102 Mtub (4, 21, 30 and 39), ETR-A and QUB (11b, 26 and 4156) were selected from amongst
103 spoligotype-defined clusters SIT53 (n= 59), SIT34 (n=15) and SIT4 (n=11) for MIRU-VNTR
104 typing after a comprehensive literature review of strains belonging to lineage 4 [16-21].

105 **2.6. MLST**

106 Classification of isolates into PGG2 or PGG3 was carried out based on polymorphism at *gyrA*95
107 codon, as reported by Sreevatsan *et al* (1997) [4]. Detailed procedures for PCR amplification of
108 *gyrA* gene and sequence comparison were according to Poudel *et al* (2013) [22]. Differentiation of
109 the isolates belonging to PPG3-SNP cluster group 6 (SCG -6) into SCG-6a and SCG-6b was carried
110 out by sequencing based on the SNP at position 311611 (T/G) of the H37Rv chromosome [5]. PCR
111 primers (311611-Fw: CCTGCACAGTGCGGTCGACG and 311611-Rv:
112 GTTCAAAGCAGCCGGCCACG) were designed to amplify a 400 bp product including the SNP
113 position. PCR and sequencing procedures were same as Poudel *et al* (2013) [22] with a primer set
114 described above.

115 **2.7. Data management and analysis**

116 Major MTBC clade/subclade spoligotype signatures and Spoligotyping International Type
117 numbers (SIT) were assigned according to the SITVIT WEB database [23] and are available online
118 at http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/. Hunter–Gaston Diversity Index

119 (HGDI) was used to determine the diversity of each MIRU–VNTR locus and the discriminatory
120 power of the typing schemes [24]. A cluster was defined as a group of two or more isolates sharing
121 the same spoligotype or MIRU-VNTR pattern, and the clustering was calculated as a ratio of
122 clustered isolates. Phylogenetic analysis and the drawing of dendrograms showing isolates
123 clustering were generated based on a clustering algorithm by the hierarchic unweighted pair group
124 method analysis (UPGMA) using an online MIRU-VNTR_{plus} database application available at:
125 <http://www.miru-vntrplus.org/> [25].

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3. Results

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3.1. Sociodemographic data analysis

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Most of isolates (83%) were recovered from treated pulmonary TB patients, and the majority
(68%) collected from Cairo and Alexandria. Other epidemiological data are shown in Table 1.

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3.2. Spoligotyping and LSPs

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The distribution of lineages/clades of MTB strains across Egyptian governorates are shown in
Table 2 and Supplementary Figure 1. All MTB isolates exhibited 76 spoligotype patterns, with 203
isolates belonging to 55 shared types and 27 showing orphan spoligotypes patterns. Only two
lineages, lineage 4 and lineages 3, were identified. The predominant lineage was lineage 4 with 214
isolates, while lineage 3 was detected in 16 isolates. The overall repartition of strains according to
major phylogenetic MTB clades was carried out based on signatures found in the SITVIT WEB
database: ill-defined T clade 102/230 (44.3%) > LAM 31/230 (13.4%) > Haarlem 25/230 (10.8%) >
S clades 17/230 (7.4%) and CAS1_Delhi 13/230 (5.6%). About 40% of all typed isolates were
found to be associated with SIT53, SIT34, SIT4, SIT25 and SIT50. LSPs showed that most orphan
strains belonged to lineage 4, and that only two were attributed to lineage 3. Phylogenetic analysis
revealed the existence of 27 clusters, the largest comprising 59 strains displaying SIT53 spoligotype
patterns, followed by two of 15 and 11 strains displaying SIT34 and SIT4 spoligotype patterns,
respectively.

145 3.3. MIRU-VNTR result analysis

146 We subjected SIT53, SIT34 and SIT4 to MIRU-VNTR typing using the 12 selected loci.
147 Eight specimens showed two or more MIRU-VNTR bands either in a single locus or several loci
148 (excluded from the phylogenetic analysis), but only 77 isolates exhibiting single MIRU-VNTR
149 band/locus were considered for further analysis. Phylogenetic analysis revealed that, of the 52
150 isolates belonging to spoligotype-defined cluster SIT53, 27 fitted into 10 clusters and the remaining
151 25 exhibited unique MIRU-VNTR patterns with a clustering rate of 52%. Out of the 15 isolates
152 belonging to SIT34, 7 were grouped into 3 clusters, and 8 showed different patterns with a
153 clustering rate of 47%. All isolates of the SIT4/unknown clade yielded diverse MIRU-VNTR
154 patterns (Figure 2). Amongst the 13 MIRU-VNTR clusters obtained, the biggest cluster consisted of
155 six isolates belonging to the SIT53/T1 clade isolated from patients from the governorate EL
156 Gharbia.

157 Individual and cumulative HGDI, detailed allelic diversity and the clustering rate for each
158 MIRU-VNTR locus are shown in Table 3 and Supplementary Table 1. The results obtained from the
159 analysis of SIT53T1, SIT34/S and SIT4 revealed that QUB-26, ETR-A and Mtub39 showed high
160 discriminatory power ($HGDI > 0.6$), whilst of the remaining loci, seven had moderate ($0.3 \leq HGDI$
161 ≤ 0.6) and two had poor ($HGDI < 0.3$) discriminatory power. In SIT53, Mtub39, QUB-26 and
162 QUB-11b yielded high discriminatory power, whilst 2 and 7 loci showed moderate and poor
163 discriminatory power, respectively. The overall discriminatory power of the MIRU-VNTR typing
164 was $HGDI = 0.9376$ in all analysed isolates versus $HGDI = 0.9145$ in SIT53.

165 3.4. MLST

166 A total of 52, 15 and 10 isolates belonging to SIT53, SIT34 and SIT4, respectively, were
167 subjected to SNP analysis of codon 95 in *gyrA*. The results showed that three isolates belonging to
168 SIT53 yielded overlapped peaks and hence, were excluded from further analysis. SIT53 was
169 differentiated into 47 AGC (Ser) and 2 ACC (Thr) corresponding to PGG3 and PGG2, respectively
170 (Figure 2A), whilst all isolates belonging to SIT34 and SIT4 were classified as PGG2 (Figure 2B).

SNP typing at position 311611 subdivided isolates belonging to SIT53-SCG6 into 46 isolates with T (SCG6a) and 1 with G (SCG6b) [5]. A previously unknown SNP at position 311614 subdivided isolates belonging to SIT53-SCG6a into 2 sub-clusters, 40 isolates with G and 6 with C (Figure 2A).

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4. Discussion

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4.1. Spoligotyping and LSPs data interpretation

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The results of the present work indicated that the majority of TB patients (93%) were infected with strains belonging to lineage 4, which reflected the actual predominance of this lineage in Egypt. Lineage 4 belongs to modern PGG2-3 and is known to be prevalent in Europe and America. Comparative analysis of prevalence of these strain families between Egypt and neighbouring EMR and other North African countries [12-14, 17, 26-31] highlights an important observation that certain strain families seem to be prevalent and widely spread in defined geographical areas. For example, Ill-defined T clade was endemic in Egypt and Syria, the Harlem family in Tunisia and the LAM family in Morocco. This observation implies that MTB exhibits clonal and phylogeographical population structures that importantly defines the geographical distribution of relevant strain-specific phenotypic traits including differences in virulence and immunogenicity [7]. Detailed comparative data are shown in Table 4.

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Previous studies conducted in Egypt have described the MTB lineages and spoligotypes circulating amongst the local population (Table 4). Cooksey *et al.*, (2002) analysed 67 clinical isolates from cerebrospinal fluid (CSF) of meningitis patients during the period 1998-2000 [12], and Abbadi *et al.*, (2009) analysed a total of 45 MTBC strains isolated from the sputum of pulmonary TB patients in the Suez Canal region of Egypt [13]. Both studies found lineage 4 to be predominant. Our results on pulmonary tuberculosis are in agreement with the above-mentioned findings regarding the prevalence of that lineage over time and geographical distribution throughout Egypt. In addition, Helal *et al.*, (2009) examined a total of 151 MTB strains from patients collected

197 over a period of one year (2005) at three different chest hospitals in Egypt [14]. However, their
198 study showed a high proportion of the ancestral MANU genotype of MTB (27.2%), which contrasts
199 with our work, as we were unable to identify any isolate belonging to MANU clade. The cause of
200 this discrepancy maybe due to MANU, a well-known spoligotype clade, can be constructed with a
201 mixture of more than two spoligotypes [32]. According to those studies, the predominance of
202 lineage 4 and the endemicity of T clade in Egyptian MTB isolates over a period of time (15 year or
203 more) indicate that these strains are strongly established, adapted and associated with the host.

204 According to our results, strains belonging to lineage 3 were found to be accounting for 7% of
205 TB morbidity in Egypt (Table 4). It was also found that about 5.6% of total isolates belonged to
206 CAS1_Delhi. An important factor affecting the prevalence of the CAS/CAS1_Delhi family in
207 Egyptian TB patients is that Egypt has deeply rooted geographical, historical and social relations
208 with many African and Asian countries like Sudan, Saudi Arabia and Iraq, from which previous
209 studies reported the predominance of CAS/CAS1_Delhi at 49% [26], 22.5% [27] and 24% [30],
210 respectively.

211 **4.2. MIRU-VNTR and clustering analysis**

212 A total of 85 isolates belonging to the largest spoligotype-defined clusters SIT53, SIT34 and
213 SIT4 were subjected to further analysis using the selected 12 loci MIRU-VNTR scheme. The SIT53
214 cluster was efficiently discriminated and subdivided into 35 MIRU-VNTR patterns. The largest
215 MIRU-VNTR-defined cluster 315422212352/SIT53 comprised of six isolates, which, according to
216 the epidemiological data, were all recovered from patients from the governorate of EL Gharbia.
217 Furthermore, the second largest cluster, 325422231452/SIT53, included three isolates and also
218 recovered from patients from the same governorate. It is worth mentioning that MTB strains
219 circulating in this governorate were seemingly transmitted from patient to patient. In contrast, all
220 isolates belonging to the spoligotype-defined SIT4 were completely discriminated from each other
221 and exhibited diverse MIRU-VNTR patterns. This observation can be explained by the fact that
222 strains belonging to the SIT4/unknown clade might have independently evolved by acquiring

223 genetic diversity over a long period of time that resulted in successively established strains stably
224 associating with the Egyptian population [9].

225 Comparative analyses were performed between our data and those reported from others
226 countries based on MIRU-VNTR typing of MTB strains belonging to SIT53 (Table 5) [18-21, 33].
227 Locus Mtub21 was highly conserved in Egyptian MTB strains belonging to SIT53 and showed poor
228 discrimination with HGDI = 0.0384 in the same manner as the isolates belonging to SIT34 and
229 SIT4 with one repeat unit. In contrast, the same locus was variable and had high and moderate
230 discriminatory power in the MTB isolates from other countries (Table 5). This result has prompted
231 for the selection and optimisation of suitable MIRU-VNTR typing schemes to achieve better
232 characterizations, discriminations and phylogenetic examinations of MTB strains based on local
233 characteristics in each geographical region. The current study is the first to optimise suitable
234 MIRU-VNTR schemes for genotyping of lineage 4, which is endemic in Egypt. According to the
235 results of the discriminatory power of the 12 selected loci shown in Table 3, the MIRU-VNTR
236 scheme that includes QUB-26, ETR-A, Mtub39, QUB-11b, MIRU16, MIRU10, Mtub04, Mtub21,
237 and MIRU40 is proposed for the analysis of isolates belonging to lineage 4, including SIT53, SIT34
238 and SIT4. For the prevalent SIT53, the MIRU-VNTR set that includes loci Mtub39, QUB-26,
239 QUB-11b, ETR-A, MIRU40, MIRU26, MIRU10 and MIRU16 is recommended for efficient
240 discrimination.

241 **4.3. MLST and PGG phylogenetic analysis.**

242 Combined data from spoligotyping, MIRU-VNTR and MLST indicate that isolates
243 determined as SIT53 were highly diverse. Two of them were belonging to a genetically distinct
244 group, PGG2, although majority were PGG3 (Figure 2). Among SIT53-PGG3 (defined as SCG6 in
245 Filliol *et al*'s SNP cluster group [5]), vast majority were SCG6a, however, one isolate was revealed
246 as SCG6b. Similar observations on the high diversity of SIT53 have been reported [5, 10, 11] and
247 this may be attributed to the definition of the spoligotype. SIT53 is defined as "all spacer present
248 except 33-36" [34, 35], i.e. the prototypic form of the lineage 4 including ill-defined T clade. Thus,

249 this classical, worldwide prevalent spoligotype strains could have higher variety in their genome
250 than other spoligotype strains emerged later. SIT53 is also known to be produced by a mixed
251 infection with different genotypes as a false spoligotype [20, 32]; however, we carefully checked
252 and excluded the samples showed multiple bands in MIRU-VNTR analysis to eliminate the possible
253 misidentification. The poor discrimination power of spoligotyping for ill-defined T clade should be
254 compensated with MIRU-VNTR and/or MLST, especially in the area where the majority of the
255 isolates show the same spoligotype like SIT53 in Egypt.

256 The isolates belonging to the biggest MIRU-VNTR cluster,
257 315422212352/SIT53-PGG3-SCG6a, showed the same characteristic SNP C at position 311614
258 (Figure 2). This cluster consisted of six isolates recovered from patients from the governorate of EL
259 Gharbia, which suggested that this clone seems to be actively circulating amongst the population of
260 that region.

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5. Conclusions

263 Our analysis demonstrated that the MTB population structure exclusively belonged to the
264 modern evolutionary phylogenetic group PGG2-3. Lineage 4 was predominant, and T family was
265 the most endemic genotype among MTB strains isolated from Egypt in 2012-2014. The genotyping
266 protocol established in the current study will contribute to efficient characterizations,
267 discriminations and phylogenetic clusters examinations based on local characteristics of MTB
268 strains circulating in Egypt. In addition, based on the results from the present study, analysis of
269 more representative number of isolates from Egypt is recommended to further elucidate the
270 prevalence, evolutionary history and actual distribution of newly found SNP at position 311614 in
271 Egyptian MTB isolates and ascertain the possible use of this SNP as a genetic marker for rapid and
272 robust discriminations of MTB isolates belonging to PGG3/SCG-6. This additional study could
273 contribute to the implementation of more effective control measures by Egyptian public health
274 authorities, which may help prevent spread of TB infection.

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Acknowledgements

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Fig 1. Geographical distribution of *M. tuberculosis* isolates from the 16 Egyptian governorates used in the present study.

Table 1. Patient epidemiological and demographic data of the present study

	Variants	Number	Ratio
Sex	Male	169	73
	Female	45	20
	No data	16	7
Governorates (patients origin)	Cairo	112	49
	Alexandria	44	19
	Others	60	26
	No data	14	6
Treatment history	Treated cases	190	83
	New cases	24	10
	No data	16	7

Table 2. Frequency distribution of major lineages/subclades of *M. tuberculosis* in Egyptian isolates.

Global lineage/Subclades *			No. of isolates	% of isolates
Lineage 1	-		0	0
Lineage 2	-		0	0
Lineage 3	CAS		14	6.1
	Orphan strains		2	0.87
Lineage 4	T superfamily	T1	89	38.7
		T2	2	0.87
		T3	6	2.6
		T4	5	2.2
	Haarlem		1	0.43
		H1	5	2.2
		H3	19	8.3
	LAM	LAM	7	3
		LAM1	1	0.43
		LAM3	2	0.87
		LAM5	1	0.43
		LAM6	1	0.43
		LAM7_TUR	8	3.5
		LAM9	9	3.9
		LAM10_CAM	2	0.87
	S		17	7.4
	Orphan strains		25	10.87
	Unknown		14	6.1
Lineage 5	-		0	0
Lineage 6	-		0	0
Lineage 7	-		0	0
Total			230	100

*Lineages classifications based on [9], [11] and [23].

Table 3 Individual and cumulative HGDI of MIRU-VNTR loci in [SIT53T1 + SIT34/S + SIT4/Unknown] versus independent SIT53/T1.

SIT53T1 + SIT34/S + SIT4/unknown clade ^a									
locus	Alias	No. of patterns	No. of clusters	No. of clustered isolates	Size of clusters	Unique patterns	Clustering rate (%)	Individual HGDI ^c	Cumulative HGDI ^c
4052	QUB-26								
2165	ETR-A	16	13	74	2 to 16	3	96.1	0.6992	0.9056
3690	Mtub39	30	19	66	2 to 9	11	85.7	0.6651	0.9586
2163b	QUB-11b	43	16	50	2 to 7	27	64.9	0.5971	0.9761
1644	MIRU16	45	16	48	2 to 6	29	62.3	0.5386	0.9798
960	MIRU10	49	14	42	2 to 6	35	54.5	0.5311	0.9829
424	Mtub04	54	14	37	2 to 6	40	48.1	0.4788	0.9870
1955	Mtub21	54	14	37	2 to 6	40	48.1	0.4617	0.9870
802	MIRU40	55	13	35	2 to 6	42	45.5	0.4392	0.9874
2996	MIRU26	56	13	34	2 to 6	43	44.2	0.3014	0.9880
4156	QUB-4156	56	13	34	2 to 6	43	44.2	0.1729	0.9880
2401	Mtub30	56	13	34	2 to 6	43	44.2	0.0513	0.9880
SIT53/T1									
3690	Mtub39							0.7375	0.7376
4052	QUB-26	15	11	48	2 to 15	4	92.3	0.6305	0.8824
2163b	QUB-11b	23	9	38	2 to 8	14	73.1	0.6041	0.9397
2165	ETR-A	27	13	38	2 to 6	14	73.1	0.5618	0.9661
802	MIRU40	29	12	35	2 to 6	17	67.3	0.3115	0.9691
2996	MIRU26	31	11	32	2 to 6	20	61.5	0.2805	0.9713
960	MIRU10	33	10	29	2 to 6	23	55.8	0.2745	0.9736
1644	MIRU16	33	10	29	2 to 6	23	55.8	0.2149	0.9736
424	Mtub04	35	10	27	2 to 6	25	51.9	0.2149	0.9774
4156	QUB-4156	35	10	27	2 to 6	25	51.9	0.1478	0.9774
2401	Mtub30	35	10	27	2 to 6	25	51.9	0.0754	0.9774
1955	Mtub21	35	10	27	2 to 6	25	51.9	0.0385	0.9774

^a SIT: Spoligotyping International Type number.^b : MIRU-VNTR: Mycobacterial Interspersed Repetitive Unit–Variable Number Tandem Repeat.^c : HGDI: Hunter–Gaston Diversity Index.

Table 4. Summary of *M. tuberculosis* lineage/clade distributions from previous studies in Egypt and other EMR[†] and North African countries

Lineages/Clades*		Country of isolation (%)										
		Egypt				Sudan	Saudi Arabia		Syria	Iraq	Tunisia	Morocco
		Current study 2015 N= 230	Cooksey et al (2002) [12] N= 67	Abbadi et al (2009) [13] N= 44	Helal et al (2009) [14] N= 151	Sharaf et al (2011) [26] N= 235	Al-Hajoj et al (2007) [27] N= 1,505	Varghese et al (2013) [28] N= 322	Bedrossian et al (2013) [29] N= 96	Merza and Salih (2012) [30] N= 53	Namouchi et al (2008) [31] N= 378	Lahlou et al (2012) [17] N= 592
Lineage 1	Manu	0	0	0	27.2	0	2.7	0	0	0	0	0
Lineage 3	CAS	7	13.6	9	4	50.7	22.5	21.1	10.4	24	0	0
Lineage 4	T	44.4	34.8	50	53.6	8.2	19.5	0	47.8	30	16.4	20.1
	Haarlem	10.9	1.5	4.5	2	3	7.5	10.6	13.4	18	40.2	22.6
	LAM	13.5	15.2	18.2	4.6	2.2	7.2	7.5	22.8	0	28.3	43.7
	S	7.4	6.1	4.5	1.3	0.4	0	2.45	0	14	0	3.8

[†] Eastern Mediterranean Region.

N: number of isolates

*Lineages classifications based on [9], [11] and [23].

Table 5 Comparison of data from the HGDI analysis based on 12 MIRU-VNTR loci used in the present study with those reported by other researchers in MTB strains belonging to SIT53/T1 ^a.

MIRU-VNTR / HGDI ^{b, c}			Country					
			Egypt 2015 N = 52	Italy Rindi <i>et al</i> (2014) [19] N = 13	Zambia Mulenga <i>et al</i> (2010) [20] N = 16	South Africa Stavrum <i>et al</i> (2009) [21] N = 13	Brazil Vasconcellos <i>et al</i> (2014) [22] N = 26	Co^ te d'Ivoire Ouassa <i>et al</i> (2012) [33] N = 74
MIRU-VNTR	3690	Mtub39	0.7375	0.7051	0.5809	0.8333	0.7169	0.0270
	4052	QUB-26	0.6305	0.5385	0.6912	0.7179	0.8338	0.0796
	2163b	QUB-11b	0.6041	0.7308	0.4853	0.7692	0.8031	0.1292
	2165	ETR-A	0.5618	0.5128	0.5294	0.3846	0.5815	0.0270
	802	MIRU40	0.3115	0.7308	0.75	0.6795	0.8092	0.4838
	2996	MIRU26	0.2805	0.7564	0.3235	0.5385	0.6092	0.0796
	960	MIRU10	0.2745	0.5897	0.5	0.6026	0.7969	0.0270
	1644	MIRU16	0.2149	0.8205	0.5294	0.5385	0.6246	0
	424	Mtub04	0.2149	0.7308	0.2279	0.5	0.6831	0.2217
	4156	QUB-4156	0.1478	0.1538	0.3824	0.4103	0.6092	0.0270
	2401	Mtub30	0.0754	0	0.4706	0.3846	0.7108	0.2455
	1955	Mtub21	0.0385	0.1538	0.4853	0.5256	0.6769	0.1292

^a SIT: Spoligotyping International Type number.

^b MIRU-VNTR: Mycobacterial Interspersed Repetitive Unit–Variable Number Tandem Repeat.

^c HGDI: Hunter–Gaston Diversity Index.

N: number of isolates

Supplementary Figure 1. UPGMA tree dendrogram showing spoligotyping patterns and clustering analysis of the 230 *Mycobacterium tuberculosis* isolates from Egypt. The categorical-based UPGMA tree was generated by an MIRU-VNTR_{plus} database application available online at (<http://www.miru-vntrplus.org/>).

Supplementary Table 1 Frequency distribution of MTB isolates [SIT53/T1 + SIT34/S + SIT4/Unknown] versus independent SIT53/T1 in the selected 12 MIRU-VNTR loci.

			SIT53/T1 + SIT34/S + SIT4/unknown clade *									
MIRU -VNTR †		No of isolates (N=77)	Number of repeats									
locus	Alias		0	1	2	3	4	5	6	7	8	9
960	MIRU10	77			14	50	11	2				
1644	MIRU16	77		46	6	25						
2996	MIRU26	77		2		4	7	64				
802	MIRU40	77		1	7	7	57	3	1	1		
424	Mtub04	77		5	53	17	2					
1955	Mtub21	77		25	51	1						
2401	Mtub30	77			75		2					
3690	Mtub39	77		6	2	39	21	5	3	1		
2165	ETR-A	77		3	26	24	24					
2163b	QUB-11b	77		1	5	31	38	2				
4052	QUB-26	77			3	9	16	31	3	11	2	2
4156	QUB-4156	77	2	3	70	2						
SIT53/T1												
MIRU -VNTR †		No of isolates (N=77)	Number of repeats									
locus	Alias		0	1	2	3	4	5	6	7	8	9
960	MIRU10	52			6	44		2				
1644	MIRU16	52		46	3	3						
2996	MIRU26	52		2		2	4	44				
802	MIRU40	52		1	5	1	43	1		1		
424	Mtub04	52		3	46	3						
1955	Mtub21	52			51	1						
2401	Mtub30	52			50		2					
3690	Mtub39	52		6	2	23	12	5	3	1		
2165	ETR-A	52		3	26	23						
2163b	QUB-11b	52		1	5	20	26					
4052	QUB-26	52			3	9	10	29			1	
4156	QUB-4156	52		2	48	2						

^a SIT: Spoligotyping International Type number.

^b : MIRU-VNTR: Mycobacterial Interspersed Repetitive Unit–Variable Number Tandem Repeat.

^c : HGDI: Hunter–Gaston Diversity Index.