JOURNAL OF CLINICAL MICROBIOLOGY, June 2009, p. 1911–1914 Vol. 47, No. 6
0095-1137/09/$08.00+0 doi:10.1128/JCM.00535-09

First Insight into *Mycobacterium tuberculosis* Epidemiology and Genetic Diversity in Trinidad and Tobago▽

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Received 16 March 2009/Returned for modification 13 April 2009/Accepted 17 April 2009

This report is based on a 1-year recruitment of all of the culture-positive *Mycobacterium tuberculosis* cases in Trinidad and Tobago ($n = 132$). The study population was characterized by a high male-to-female sex ratio of 4 and a human immunodeficiency virus-tuberculosis (TB) coinfection rate of 30%. It mainly occurred among African descendants, who represent 37.5% of the total population but 69.7% of all TB cases ($P < 0.001$). Spoligotyping resulted in 25 different patterns and 12 clusters (2 to 74 strains per cluster), with the predominance of a highly conserved spoligotype international type clone, SIT566.

Referred to as the business capital of the Caribbean, Trinidad and Tobago (T&T) had an annual tuberculosis (TB) incidence rate of 17 cases/100,000 population in 2006, and control of TB remains a priority in T&T (6). The number of reported cases has remained stable over the last decade, with an average of 196 cases per year (range, 159 to 253) and in 2006, the majority of the cases were seen in the County of St. George, with more than one-half of the reported cases. Although *Mycobacterium tuberculosis* genetic biodiversity was recently studied in the French departments of the Americas and Haiti (3, 5), the present study conducted in T&T is the first among all English-speaking Caribbean islands to study the genetic diversity of circulating *M. tuberculosis* clones.

All of the 132 TB patients presenting a positive *M. tuberculosis* culture from October 2006 to September 2007 were included in the present study, and basic demographic data were collected by using a standard questionnaire from the files provided at the hospital. Spoligotyping was carried out as previously described (7) on bacterial DNA samples shipped to the Pasteur Institute of Guadeloupe, and the patterns obtained were compared by using the SITVIT2 proprietary database of the Pasteur Institute of Guadeloupe, which is an updated version of the previously released SpolDB4 database (2). In this database, SIT (spoligotype international type) designates spoligotyping shared by two or more patient isolates, whereas MIT (mycobacterial interspersed repetitive unit [MIRU] international type) designates 12-locus MIRU patterns shared by two or more patient isolates, as opposed to "orphan," which designates patterns reported for a single isolate. Major phylogenetic clades were assigned according to the signatures provided in SpolDB4 (2). Lastly, PCR-based 12-locus MIRU typing was performed on selected DNA samples by using primers described previously (9, 13).

Compared to the extremely high annual TB incidence in some hot spots in the Caribbean such as Haiti (299/100,000) and the Dominican Republic (89/100,000) (http://www.who.int/globalatlas/dataQuery/default.asp), the TB incidence in T&T remained moderate at 17/100,000 in 2006 (6). However, the TB patients in T&T were characterized by a very high male-to-female sex ratio of 4.0, which is significantly higher than the sex ratios observed in Martinique (1.0), Haiti (1.2), and Guadeloupe (2.1) ($P < 0.001$ for Martinique and Haiti, $P < 0.02$ for Guadeloupe) (3, 6). African descendants represented 37.5% of the total population but 69.7% of all TB cases ($P < 0.001$). Of the 119 patients whose case statuses were known, 77.3% (92/119) were new cases and 22.7% (27/119) belonged to the category of treatment failure/relapse/defaulter. The study population (mean age, 42.8 years; range, 17 to 78 years; 54/125 cases in the 35-to-54-year age group, $P < 0.05$) was characterized by a human immunodeficiency virus (HIV)-TB coinfection rate of 30.6% (HIV serology results were available for 121/132 patients). No cases of multidrug-resistant TB (combined resistance to isoniazid and rifampin) were observed, a finding which is in contrast to previous studies in Africa and the Caribbean, where high HIV-TB coinfection rates were often accompanied by the emergence of multidrug-resistant TB (4, 5, 8, 12). Regarding the geographic origin of the patients, 40.5% (15/37) of the HIV-positive patients versus 17.9% (15/84) of the HIV-negative patients lived in central St. George county, which includes the capital city of Port of Spain ($P < 0.01$).

Spoligotyping resulted in 25 patterns corresponding to 13 single isolates and 12 clusters (2 to 74 strains per cluster) (Table 1). A high clustering rate of 90% (119/132 isolates) was due to a single large cluster of 74 strains (SIT566) along with five other major clusters (SIT1, SIT61, SIT2550, and two newly created shared-types, SIT2934, SIT2935; 5 to 8 strains/cluster). The six remaining smaller clusters (SIT42, 50, SIT119, 478, 958, and 1823) contained two or three strains per cluster. Among the 13 unclustered strains, 12 belonged to preexisting

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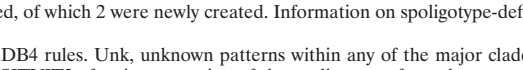
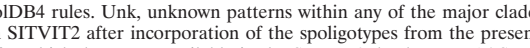
▽ Published ahead of print on 29 April 2009.

TABLE 1. Spoligotyping-based biodiversity in T&T and SITVIT2 database comparison[a]

SIT	Binary spoligotype pattern	Clade[b]
566		Unk
1710		LAM
61		LAM10-CAM
2550		LAM10-CAM var
17		LAM2
60		LAM4
93		LAM5
64		LAM6
42		LAM9
126		EAI
138		EAI
958		EAI1-SOM var
2934[e]		EAI2-Manilla var
2935[e]		EAI2-Manilla var
119		X1
1823		X1 var
478		X2 var
70		X3 var
1		Beijing
73		T
53		T1
50		Haarlem 3
288		CAS2
450		Unk
orphan		AFRI-2 var

[a] Shown are all 25 of the patterns observed, of which 2 were newly created. Information on spoligotype-defined lineages and distribution in other Caribbean settings is also shown.

[b] Clades were assigned by following SpolDB4 rules. Unk, unknown patterns within any of the major clades described in SITVIT2; var, variant.

[c] Shown is the number (%) of strains in SITVIT2 after incorporation of the spoligotypes from the present study ($n = 132$).

[d] Distribution in neighboring countries for which data were available in the SITVIT2 database on 15 September 2008. The three-letter codes for Cuba ($n = 256$), Guadeloupe ($n = 342$), French Guiana ($n = 566$), Haiti ($n = 404$), Martinique ($n = 158$), and Venezuela ($n = 927$) are according to http://en.wikipedia.org/wiki/ISO_3166-1_alpha-3.

[e] Concerns new SITs created after a match within this study (SIT2934) or with another orphan strain in SITVIT2 (SIT2935).

SIT designations in the database, while a single isolate corresponded to an orphan pattern. The genotypic lineage determination showed that 43.2% (57/132) of the clinical isolates were correctly classified (Table 1). The predominant clone, SIT566, and Latin American-Mediterranean (LAM) and East African-Indian (EAI) lineage strains were found throughout the country (with the exception of LAM and EAI in the eastern county of St. Andrew/St. David and LAM on Tobago). The patients harboring the SIT566 clone (versus other spoligotypes) were more often HIV positive (62.2% of the HIV-TB-coinfected patients), were significantly younger (mean age, 39.1 years versus 47.7 years, $P < 0.0005$), and mostly originated from central St. George County (31.4% of all SIT566 patients, $P < 0.05$).

Looking into the distribution of LAM lineage clones, SIT17, SIT20, and SIT93, commonly found in the Caribbean in the SITVIT2 database, were absent from T&T. On the other hand, the LAM-10CAM lineage is so far limited only to T&T (present with its prototype SIT61 [$n = 6/132$] and a variant, SIT2550 [$n = 7/132$]). This sublineage is phylogeographically specific for Cameroon and neighboring countries in West Africa (2, 10), and interestingly, 75% of the patients belonging to this group in our study were of African descent. We also observed a very low proportion (1.5%) of the Haarlem lineage strains of European descent in T&T, although they represented around 25% of the strains found in Guadeloupe, Martinique, and French Guiana (2, 3). On the contrary, T&T shares with its French-speaking Caribbean neighbors (Haiti, Guadeloupe, and Martinique) the specific presence of the X lineage known to have phylogeographic specificity for Anglo-Saxon descendants (2, 11). Lastly, we found six strains of the Beijing family, the majority being isolated from patients below 45 years of age. Beijing strains were previously associated with mostly younger persons in Vietnam and were thought to be an indicator of recent and ongoing transmission (1).

Despite its limitations when used alone (11), spoligotyping provided a reasonable estimate of the population structure in our setting; e.g., excluding the SIT566 clone, the diversity observed was composed of 11 clusters (45/132 or 34.1% of the strains) belonging to five major lineages: EAI (15 of the 45 clustered isolates), LAM (15/45), X (7/45), Beijing (6/45), and Haarlem (2/45). EAI and LAM isolates each represented one-third of the clustered isolates. The presence of these lineages underlines the origin of the population and the demographic history of T&T, with historical and persistent links with the (i) Indian subcontinent and (ii) Latin America and the Mediterranean region, respectively, where these lineages predominate (2). In summary, the *M. tuberculosis* population structure in T&T does not resemble that of other Caribbean neighbors. It may, however, be noted that data in the SITVIT2 database are representative of Guadeloupe, Martinique, and French Guiana over a 15-year period, 1994 to 2008, but represent a much shorter recruitment period for the remaining countries, including T&T. This limitation must be kept in mind when drawing

TABLE 1—*Continued*

No. (%) of strains in:		% in this study compared to SITVIT2	Distribution in neighboring countries[d]
This study	SITVIT2[c]		
74 (56)	86 (0.15)	86.05	Absent
1 (0.8)	6 (0.01)	16.67	VEN (1)
6 (4.5)	618 (1.07)	0.97	GLP (1), GUF (1), MTQ (1)
7 (5.3)	14 (0.02)	50	Absent
1 (0.8)	540 (0.94)	0.19	CUB (1), GLP (17), GUF (8), HTI (22), MTQ (3), VEN (179)
1 (0.8)	207 (0.36)	0.48	CUB (4), GUF (2), VEN (15)
1 (0.8)	295 (0.51)	0.34	GLP (16), GUF (4), HTI (15), VEN (94)
1 (0.8)	260 (0.45)	0.38	GUF (19), MTQ (1), VEN (4)
2 (1.5)	2,151 (3.74)	0.09	CUB (19), GLP (22), GUF (17), HTI (27), MTQ (4), VEN (111)
1 (0.8)	90 (0.16)	1.11	Absent
1 (0.8)	95 (0.16)	1.05	GLP (1)
2 (1.5)	15 (0.03)	13.33	GUF (2)
8 (6)	8 (0.01)	100	Absent
5 (3.8)	6 (0.01)	83.33	Absent
3 (2.3)	962 (1.67)	0.31	GUF (1), HTI (1), MTQ (3)
2 (1.5)	4 (0.01)	50	Absent
2 (1.5)	26 (0.05)	7.69	Absent
1 (0.8)	101 (0.18)	0.99	GLP (3), GUF (6), HTI (8)
6 (4.5)	6,078 (10.6)	0.1	CUB (26), GLP (1), GUF (8), HTI (1), MTQ (3), VEN (2)
1 (0.8)	173 (0.30)	0.58	GLP (1), GUF (7), HTI (1), MTQ (1), VEN(1)
1 (0.8)	4,113 (7.14)	0.02	CUB (23), GLP (32), GUF (73), HTI (27), MTQ (21), VEN (49)
2 (1.5)	2,376 (4.13)	0.08	CUB (22), GLP (21), GUF (77), HTI (37), MTQ (15), VEN (13)
1 (0.8)	86 (0.15)	1.16	Absent
1 (0.8)	73 (0.13)	1.37	Absent
1 (0.8)	1 (0.001)	100	Absent

conclusions about the overall population structure in the Caribbean.

Regarding the worldwide distribution of predominant clone SIT566, only 86 strains were present in the database and all but 12 belonged to this study (Table 1); 11 were from the United States (J. Driscoll, personal communication), and 1 was from T&T (not included in the present study). The patient origin was known for 6/11 strains isolated in the United States, and all six of the patients were T&T born, indicating that these *M. tuberculosis* strains did originate in T&T. We attempted 12-locus MIRU typing of eight randomly chosen strains, which led to the same 12-locus pattern of 224315153324. In the SITVIT2 database, this pattern corresponded to a rare shared type designated MIT633, with only 12 strains with the following distribution: Peru, $n = 1$; United Kingdom, $n = 2$; United States, $n = 9$. Spoligotyping data were available in parallel for 10/12 strains. Interestingly, all five of the isolates from the United States were found to be strictly identical to SIT566, i.e., lacking spacers 14 to 18, 33 to 36, and 40. The remaining five isolates shared with SIT566 the absence of spacers 33 to 36 (which is characteristic of evolutionarily modern *M. tuberculosis* lineages [2, 11]) and possessed spoligotypes evolutionarily related to the T&T clone. One strain classified as SIT219 (with the same pattern as SIT566 but with spacer 40 present) was isolated in the United States and could be tentatively classified as a potential ancestor of SIT566. The next related pattern was also that of a strain isolated in the United States (SIT2474) that had the same pattern as SIT566 plus the additional absence of spacer 38. We also observed three other patterns that could also be remotely related to the T&T clone at the evolutionary level (SIT478, SIT554, and an orphan). The important rate of clustering observed in T&T is suggestive of ongoing TB transmission with the presence of a highly conserved clone (SIT566) yet without any evidence of transmission to neighboring Caribbean islands. Whether this clone expanded recently due to a selection factor such as a higher degree of virulence of the strain or due to undetermined risk factors, will now be investigated in greater detail by using extended 24-locus MIRU typing (14).

We are indebted to the Caribbean Epidemiology Center, T&T, particularly to Ashok Rattan, Medical Microbiologist, and José Campione-Piccardo, Director, for all of their help and support in this investigation and to Lorraine Francis for technical assistance. We thank Anton Cumberbatch, Chief Medical Officer, T&T, for granting permission to use the *M. tuberculosis* isolates in this study. We also acknowledge the assistance of the staff of the Chest Clinics at the Eric Williams Medical Sciences Complex, the San Fernando General Hospital, and the Trinidad Public Health Laboratory for data collection. N.R. and J.M. thank J. Driscoll for providing information regarding the SIT566 clone in the United States.

S.B. is grateful to the University of the West Indies for financial support. The work done at the Pasteur Institute of Guadeloupe was financed by the European Regional Development Fund, European Commission (ERDF/FEDER, A34-05), and the Regional Council of Guadeloupe (CR/08-1612: Biodiversité et Risque Infectieux dans les modèles insulaires). J.M. was awarded a Ph.D. fellowship by the European Social Funds through the Regional Council of Guadeloupe.

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