

## Spoligotype Profile of *Mycobacterium tuberculosis* Complex Strains from HIV-Positive and -Negative Patients in Nigeria: a Comparative Analysis<sup>∇</sup>

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We ran a comparative analysis of all patients for whom a positive culture of *Mycobacterium tuberculosis* complex was available between April 2004 and October 2005 and whose HIV serology results were known, with spoligotyping results ( $n = 163$ ) split into 49 HIV-positive patients and 114 HIV-negative patients. Spoligotype international type 373 (SIT373) (T1 lineage), which was highly prevalent among the HIV<sup>+</sup> patients, was totally absent from the HIV<sup>-</sup> population, suggesting that we had a specific clone affecting nearly 1/3 of all HIV-tuberculosis (TB)-coinfecting patients. Among the LAM10-CAM sublineage strains, we had only a single strain of SIT403 among HIV<sup>-</sup> patients (0.88%), as opposed to 12.25% of the HIV<sup>+</sup> population ( $\chi^2 = 10.77$ ;  $P < 0.01$ ), indicating a strong association between the strain and the HIV<sup>+</sup> population. The LAM10-CAM lineage spoligotype SIT61 was prevalent among the 2 subsets (37.72% in HIV<sup>-</sup> versus 12.24% in HIV<sup>+</sup> populations), though, with a significant difference between the 2 groups ( $\chi^2 = 10.53$ ;  $P < 0.01$ ). However, there was no significant difference for SIT53 (T1 lineage) in the 2 subsets: 6.14 versus 8.2% ( $\chi^2 = 0.22$ ;  $P > 0.05$ ). A total of 7/49, or 14.3%, other SITs among HIV<sup>+</sup> patients were not found among the HIV<sup>-</sup> patients. When added to the most prevalent SIT among HIV<sup>+</sup> patients (SIT373;  $n = 16$ ), 23/49, or 47%, isolates among HIV-TB-coinfecting patients were unique. We conclude that further studies should be carried out to investigate the evolution of these genotypes and others in the emergence of multidrug resistance and control of tuberculosis in Nigeria.

With a population of over 140 million, Nigeria is faced with a huge burden of tuberculosis (TB) and is currently rated fourth among the TB-burdened nations of the world, with a prevalence of 521 per 100,000 population in the year 2007 (27). This situation is worsened by the country's HIV/AIDS epidemic, with a HIV prevalence in 2007 of 3.6% and a TB-HIV coinfection rate of 27% (25). Although the outcome of treatment was not evaluated for a high proportion of patients in 2007, the treatment success rate was 76%. Collaborative TB-HIV activities are also being scaled up, and 32% of TB cases are now screened for HIV (27). Nonetheless, the case detection rate of new smear-positive TB cases in Nigeria, which was 18% in 2005 (23% in 2007), remained unacceptably low (<http://www.stoptb.org/assets/documents/countries/GlobalReport2009/nga.pdf>). Unfortunately, only limited information is available on the genotypes of the *Mycobacterium tuberculosis* complex (MTC) isolated from Nigeria in comparison with those obtained globally, an important factor that is useful for understanding the global spread and

phylogeographical specificity of predominant circulating clones of tubercle bacilli. In addition, molecular data obtained from the MTC so far in Nigeria have not addressed the issue of TB-HIV-coinfecting patients, a subpopulation involved in the spread of the disease in Africa in general and Nigeria in particular.

Molecular typing techniques have been extensively used to speciate strains of incriminating MTC involved in TB infections; among these, spoligotyping, a method based on PCR amplification of a highly polymorphic direct-repeat locus in the MTC genome, has been extensively used for simultaneous detection and typing of these organisms (16). Indeed, due to its clonal structure (24), the comparative genotype analysis of MTCs from different human populations can give unique insights into the dissemination dynamics and evolutionary genetics of this pathogen (22); consequently, spoligotyping has been used to understand the emerging problem of multidrug-resistant (MDR) TB and the virulence of certain epidemic strains of *M. tuberculosis* (such as the Beijing strain), as well as to better comprehend the epidemiology of TB and TB-HIV coinfection (1–6, 13, 14). A previous study on molecular analysis of MTC in Nigeria did not address the issue of TB-HIV coinfection (9). The present study, therefore, aimed to investigate the spoligotyping-based population structure of MTC clinical isolates in HIV<sup>+</sup> and HIV<sup>-</sup> patients in Nigeria and to compare the patterns obtained with those available in the SITVIT2 proprietary database of the Pasteur Institute of Guadeloupe,

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TABLE 3. Description of clustered *M. tuberculosis* complex isolates (2 or more isolates in this study) from HIV+ and HIV- patients in Ibadan, Nigeria, and their worldwide distribution in the SITVIT2 database

SIT (clade) <sup>a</sup>	Total (%) in subgroup	% in subgroup vs. SITVIT2	Distribution (%) in regions with:	
			≥5% of a given SIT <sup>b</sup>	≥5% of a given SIT <sup>c</sup>
Clustered isolates in the HIV <sup>+</sup> patient subgroup (n = 49)				
<b>53 (T1)</b>	4 (8.2)	0.1	AMER-N, 19.95; AMER-S, 14.67; EURO-W, 13.0; EURO-S, 10.16; ASIA-W, 8.81; AFRI-S, 6.04	USA, 17.56; ZAF, 5.89; ITA, 5.20
<b>61 (LAM10-CAM)</b>	6 (12.2)	0.9	AFRI-M, 27.59; AFRI-W, 25.39; AMER-N, 12.54; EURO-W, 10.97; ASIA-W, 6.90	CMR, 26.49; NGA, 22.57; USA, 12.54; SAU, 6.90; FRA, 6.27
373 (T1)	16 (32.7)	29.1	AFRI-W, 29.09; EURO-W, 16.36; AFRI-S, 14.55; AMER-S, 10.91; EURO-S, 9.09; EURO-E, 9.09; AMER-N, 7.27	NGA, 29.09; ZAF, 14.55; CZE, 9.09; USA, 7.27; BEL, 7.27; PER, 5.45; AUT, 5.45; BRA, 5.45
403 (LAM10-CAM)	6 (12.2)	17.6	AFRI-M, 44.12; AFRI-W, 26.47; AMER-N, 8.82; EURO-W, 8.82; ASIA-S, 5.88	CMR, 44.12; NGA, 26.47; USA, 8.82; IND, 5.88
Clustered isolates in the HIV <sup>-</sup> patient subgroup (n = 114)				
42 (LAM9)	2 (1.75)	0.08	AMER-S, 29.83; AMER-N, 16.28; EURO-S, 12.78; EURO-W, 7.02; AFRI-N, 5.07	USA, 15.24; BRA, 10.33; COL, 7.87; ITA, 6.72
<b>53 (T1)</b>	7 (6.14)	0.15	AMER-N, 19.95; AMER-S, 14.67; EURO-W, 13.0; EURO-S, 10.16; ASIA-W, 8.81; AFRI-S, 6.04	USA, 17.56; ZAF, 5.89; ITA, 5.20
<b>61 (LAM10-CAM)</b>	43 (37.72)	6.36	AFRI-M, 27.59; AFRI-W, 25.39; AMER-N, 12.54; EURO-W, 10.97; ASIA-W, 6.90	CMR, 26.49; NGA, 22.57; USA, 12.54; SAU, 6.90; FRA, 6.27
181 (AFRI_1)	2 (1.75)	0.60	AFRI-W, 80.06; AMER-N, 6.34; EURO-W, 6.04	GNB, 51.06; GMB, 25.38; USA, 6.34
320 (AFRI_2)	2 (1.75)	22.22	AFRI-W, 44.44; AMER-N, 22.22; AFRI-M, 22.22; EURO-S, 11.11	NGA, 44.44; USA, 22.22; CMR, 22.22; ESP, 11.11
330 (AFRI_2)	2 (1.75)	33.33	AFRI-W, 33.33; AFRI-M, 33.33; EURO-W, 16.67; AFRI-N, 16.67	NGA, 33.33; CAF, 16.67; CMR, 16.67; TUN, 16.67; BEL, 16.67
331 (AFRI_2)	5 (4.39)	11.90	AFRI-W, 64.29; AMER-N, 23.81; AFRI-M, 7.14	NGA, 54.76; USA, 23.81; CIV, 7.14; CMR, 7.14
342 (EAI5)	2 (1.75)	12.50	AFRI-W, 43.75; EURO-W, 25.00; EURO-N, 18.75; AMER-N, 12.50	NGA, 31.25; GBR, 12.50; GMB, 12.50; USA, 12.50; BEL, 12.50; NLD, 6.25; DNK, 6.25; DEU, 6.25
438 (AFRI_3)	4 (3.51)	50.00	AFRI-W, 75.00; AMER-N, 25.00	NGA, 62.50; USA, 25.00; SLE, 12.50
504 (T3)	2 (1.75)	15.38	AMER-N, 53.85; AFRI-W, 15.38; EURO-W, 7.69; EURO-S, 7.69; ASIA-S, 7.69; AFRI-E, 7.69	USA, 53.85; NGA, 15.38; TZA, 7.69; FXX, 7.69; IND, 7.69; ITA, 7.69
<b>1592 (AFRI_2)</b>	2 (1.75)	33.33	AFRI-W, 66.67; EURO-W, 33.33	NGA, 66.67; DEU, 16.67; FRA, 16.67
2550 (LAM10-CAM)	4 (3.51)	26.67	CARI, 46.67; AFRI-W, 26.67; ASIA-W, 6.67; AMER-S, 6.67; AMER-N, 6.67; AFRI-E, 6.67	TTO, 46.67; NGA, 26.67; USA, 6.67; BRA, 6.67; TUR, 6.67; MDG, 6.67
2823 <sup>d</sup> (AFRI_3)	2 (1.75)	66.67	AFRI-W, 100.00	NGA, 100.00
2825 <sup>d</sup> (LAM10-CAM)	2 (1.75)	100.00	AFRI-W, 100.00	NGA, 100.00
2829 <sup>d</sup> (AFRI_2)	2 (1.75)	100.00	AFRI-W, 100.00	NGA, 100.00
2831 <sup>d</sup> (LAM10-CAM)	3 (2.63)	100.00	AFRI-W, 100.00	NGA, 100.00
2835 <sup>d</sup> (EAI)	3 (2.63)	75.00	AFRI-W, 100.00	NGA, 100.00

<sup>a</sup> SITs simultaneously present in HIV-positive and HIV-negative subgroups are in boldface.

<sup>b</sup> Worldwide distribution is reported for regions with ≥5% of a given SIT compared to the total number in the SITVIT2 database. The definitions of regions and subregions are according to those of the United Nations (<http://unstats.un.org/unsd/methods/m49/m49regin.htm>): regions, AFRI (Africa), AMER (Americas), ASIA (Asia), EURO (Europe), and OCE (Oceania), subdivided into E (eastern), M (middle), C (central), N (northern), S (southern), SE (southeastern), and W (western). Furthermore, CARIB (Caribbean) belongs to the Americas, while Oceania is subdivided into 4 subregions, AUST (Australasia), MEL (Melanesia), MIC (Micronesia), and POLY (Polynesia). Note that in our classification scheme, Russia has been assigned a new subregion by itself (Northern Asia) instead of including it with the rest of Eastern Europe. This reflects its geographical location, as well as the similarity of specific TB genotypes circulating in Russia (a majority of Beijing genotypes) to those prevalent in Central, Eastern, and Southeast Asia.

<sup>c</sup> The 3-letter country codes are defined in wikipedia ([http://en.wikipedia.org/wiki/ISO\\_3166-1\\_alpha-3](http://en.wikipedia.org/wiki/ISO_3166-1_alpha-3)); countrywide distribution is shown only for SITs with ≥5% compared to their total number in the SITVIT2 database.

<sup>d</sup> Newly created shared type (for details, see note *d* to Table 2).

(6/13 LAM10-CAM strains in the HIV<sup>+</sup> subgroup and 43/59 strains in the HIV<sup>-</sup> subgroup), and related strains clearly derived from it, e.g., SIT115, which evolved from the prototype SIT61 by loss of spacer 15 (a single strain in the HIV<sup>+</sup> subset),

and SIT403, which evolved by loss of spacer 40 (6/13 LAM10-CAM strains in the HIV<sup>+</sup> subgroup and 1/59 in the HIV<sup>-</sup> subgroup).

In the HIV<sup>-</sup> subset, there were many other strains of the

LAM10-CAM sublineage derived from SIT61 that evolved by loss of one or more spacers (SIT838, -2550, -2825, -2830, -2831, -2832, and -2834). Among the LAM10-CAM members, we had only a single strain of SIT403 from the HIV<sup>-</sup> patients (1/114, or 0.88%), as opposed to 6/49, or 12.25%, in the HIV<sup>+</sup> population, suggesting a strong correlation between the strain and the HIV<sup>+</sup> subset. On the other hand, the LAM10-CAM lineage spoligotype SIT61 was prevalent in the 2 subsets (43/114, or 37.72%, in the HIV<sup>-</sup> population versus 6/49, or 12.24%, in the HIV<sup>+</sup> population). This was also true for SIT53 (T1 lineage) (6.14% versus 8.2%). Similarly, the T1 sublineage was represented by its prototype, SIT53 ( $n = 4$ ), but the major prevailing spoligotype among the HIV<sup>+</sup> patients, SIT373, apparently derived from the prototype by loss of spacer 24 ( $n = 16$ ), as well as a minor pattern, SIT1580 (cumulative loss of spacers 23 and 24;  $n = 1$ ). As can be seen, SIT373 (T1 lineage), which was highly prevalent among HIV<sup>+</sup> patients, was totally absent from the HIV-negative population, suggesting that we had a specific clone affecting nearly 1/3 of all HIV-TB-coinfected patients in our setting.

Another interesting feature was the smaller presence of *M. africanum* in the HIV<sup>+</sup> population (4/49, or 8.2%); nonetheless, it is the first reported case of this kind among TB-HIV-coinfected patients in Nigeria. Three of the strains were similar (i.e., they lacked spacers 7 to 16 and had most of the spacers from 25 to 36 intact). The HIV<sup>-</sup> subgroup was characterized by a larger presence of this genotype (27/114, or 23.7%). The preponderance of *M. africanum* in the HIV<sup>-</sup> subset could be seen from the presence of 3 main sublineages (AFRI-1, AFRI-2, and AFRI-3), with the occurrence of two “orphan” strains. The presence of this clade is not surprising based on earlier findings about TB patients in Nigeria (9) and other West African countries (11, 20, 21). Similar reports about this sublineage have also been made in some East African countries, though with phenotypic traits linked to *M. tuberculosis* (4, 6, 18, 19).

Quite interestingly, two strains of *M. bovis* were found in the HIV<sup>-</sup> population, with none identified in the HIV<sup>+</sup> subset, though this reflects the zoonotic transmission of the disease in humans, judging from the high prevalence of bovine tuberculosis in Nigeria (10). It is noteworthy, however, that there is a high potential for human infection with this genotype, judging from the lack of a control and eradication program for bovine tuberculosis in Nigeria, where people still consume unpasteurized milk and milk products, along with other risk factors (8, 9).

In our study, the Haarlem clade (specifically, the H3 sublineage) also featured in both subsets, making up a very small percentage of the entire data set (i.e., 1/49, or 2%, and 4/114, or 3.5%, respectively, in the HIV<sup>+</sup> and HIV<sup>-</sup> groups); however, with a majority (3/5, or 60%) of the strains being orphans and restricted to Nigeria, not much can be concluded about its significance. Again, strains of the ancestral EAI clade (4), which are very characteristic in terms of their spoligotype patterns and the presence of genomic regions of difference (with both TbD1 and RD9 not deleted), were isolated from the HIV<sup>-</sup> subset. However, the smaller percentage of these strains in the HIV<sup>-</sup> subset (5/114, or 4.4%) may explain its absence in the HIV<sup>+</sup> population in our data set. EAI represents the most ancestral clade of the *M. tuberculosis* complex (except for the

*M. canettii* ancestor [12]). Recently, an EAI5 strain was isolated from a slaughtered female goat in an abattoir within the same study area (7). Whether this animal infection resulted from cross-infection due to close human and animal cohabitation is not known, since this was the only report of its kind in our setting.

Another remarkable finding was the presence of 3 ancestral Manu lineage strains limited to orphan patterns in the HIV<sup>+</sup> population (2 were classified as Manu2 and 1 as Manu3). Incidentally, no Manu strain was found in the HIV<sup>-</sup> subgroup. The Manu lineage was initially described as a new family from India in 2004 (23), and later, similar strains in small numbers were reported in a study from Madagascar (13). Soon afterwards, it was tentatively subdivided into the Manu1 (deletion of spacer 34), Manu2 (deletion of spacers 33 and 34), and Manu3 (deletion of spacers 34 to 36) sublineages, and it was suggested that it could represent an ancestral clone of principal genetic group (PGG) 1 strains (4). Manu lineage strains were reported from Saudi Arabia (2) and Tunisia (17) and more recently in a study from Egypt, where it represented as much as 27% of all isolates (15). However, according to a recent report, a fraction of Manu2 patterns could also arise due to mixed infection by a strain belonging to evolutionarily recent H, LAM, X, and T lineages with a Beijing strain, a risk that may be excluded by looking for genomic deletion of region of difference RD105, which is characteristic of the Beijing lineage (26). However, the complete absence of Beijing strains in our setting argues against this possibility. Nonetheless, the presence of the ancestral Manu lineage strains, supposed to be a missing link from the split between ancestral and modern tubercle bacilli during *M. tuberculosis* evolution (15), should be further investigated within larger data sets to discover the contribution of Africa to the origin, evolution, and spread of tuberculosis.

In conclusion, our study shows that TB among HIV<sup>+</sup> and HIV<sup>-</sup> patients in Ibadan, southwestern Nigeria, is essentially caused by predominant genotypes characteristic of the western and middle African regions and, more specifically, the LAM10-CAM sublineage, which is also the predominant spoligotype in the neighboring countries of Cameroon and Benin. In the same vein, the *M. africanum* clade was found to play a prominent role in both subsets, a scenario that had been reported earlier in neighboring West African countries, providing further incentive to carry out detailed investigations of the clade in Nigeria. The presence of the EAI clade essentially in the HIV<sup>-</sup> population provided more insight into the diversity of the *M. tuberculosis* complex strains in Nigeria; coupled with this is the identification of the Haarlem genotype in both subsets, a situation that indicates the interaction of Nigerians with people from other countries, possibly due to trade links and the presence of Indo-Asians in Nigeria. Nonetheless, the complete absence of the Beijing genotype in our study population as opposed to a recent report from Benin (1) is noteworthy. Our study provides a good platform to work through the diversity of *M. tuberculosis* complex strains among TB patients in Nigeria, providing a basis for further epidemiological investigations into the evolution and control of TB in the country.

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