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Molecular epidemiology and genetic diversity of *Mycobacterium tuberculosis* complex in referral health centers of Bamako, Mali: What is new?☆

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Abstract

Background and Aims: Tuberculosis (TB) remains an important global health issue worldwide. Despite this scourge threatening many human lives, especially in developing countries, thus far, no advanced molecular epidemiology study using recent and more accurate tools has been conducted in Mali. Therefore, this study aimed to use variable-number tandem repeats of mycobacterial interspersed repetitive units (MIRU-VNTR) technology coupled with the spoligotyping method to accurately determine the hot spots and establish the epidemiological transmission links of TB in Bamako, Mali.

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Conceived and designed the experiments: BK, MM, AMS, MK, SeD, and SD. Performed the experiments: BK, MK, BD, AK, DD, FC, MS, ACGT, and NC. Analyzed the data: BK, MM, BD, AMS, MK, YDS, SYD, SOD, MS, RM, MD, DD, ACGT, and AK. Contributed reagents MM, RM, MD, and SS. Wrote the article: BK, AMS, MM, JLH, SeD, and BB. Reviewed by all authors.

Conflict of interest

None of the authors has a conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.01.061.

Methods: In a cross-sectional study, 245 isolates of *Mycobacterium tuberculosis* complex (MTBC) were characterized using spoligotyping and MIRU-VNTR, and an epidemiological investigation was conducted.

Results: Of the 245 isolates, 184 (75.1%) were formally identified. The most widespread strain was the Cameroon strain (83; 45.1%). Eight major clusters were identified: Ghana (27; 14.7%), West African 2 (22; 12%), Haarlem (13; 7.1%), H37Rv (t) (8; 4.3%), Latin American Mediterranean (8; 4.3%), and Uganda I and II (6; 3.3%). Statistical analysis showed a significant difference between lineages from the respective referral health centers of Bamako, Mali ($P = 0.01$).

Conclusion: This study establishes, for the first time, an accurate spatial distribution of circulating MTB strains in Bamako, Mali. The data was used to identify strains and "hot spots" causing TB infection and can also be used for more targeted public health responses, particularly for hot spots of drug-resistant strains.

Keywords

TB; Genetic diversity; Spoligotyping; MIRU-VNTR; Mali

Introduction

Mycobacterium tuberculosis (MTB) remains a major global health problem with an estimated 10.4 million new cases of tuberculosis (TB) each year, causing approximately 1.5 million human deaths worldwide, with 95% of deaths occurring in developing countries (WHO, 2020). According to the World Health Organization (WHO), regions of Southeast Asia, Africa, and the Western Pacific are experiencing the highest number of TB cases (Chakaya et al., 2021), whereas, India (26%), Indonesia (8.5%), China (8.4%), Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%), and South Africa (3.6%) are accounting for two-thirds of the global TB cases. Despite the fact that the WHO African region accounted 14% of the world's population, it was found out that a quarter of the global TB incidence occurs in the continent and possesses the highest HIV-associated comorbidity with increased fatality rates (Dye et al., 2006). Thus, sub-Saharan region in Africa is the most affected region in terms of case per population ratio. In West Africa, 3 countries (Nigeria, Liberia, and Sierra Leone) are the most affected by TB and listed among the 30 high-burden countries worldwide (Chakaya et al., 2021). Mali, located in West Africa, also has high incidences and is at a potential risk of increased rates and resistances, given that it is only in West Africa where all 6 major human TB lineages are confirmed to be present (de Jong et al., 2008 Yeboah-Manu et al., 2016). The Euro-American lineage (lineage 4) and the *M. africanum* lineages (lineage 5 and 6) are the strains that are most associated with causing pulmonary TB in West Africa (Gehre et al., 2016). According to the 2019 Mali National TB Program Report, the mean incidence of TB in Mali was 53/100,000 habitants (ranking from 90 to 117 per 100,000 habitants) (CDC Division of Global HIV and TB Country Profile, 2018). The T1 family and Latin American Mediterranean 10 (LAM-10) from lineage 4 and *M. africanum* 2 from lineage 6 were the most predominant strains reported in the Bamako region (Togo et al., 2017).

Molecular epidemiology of MTB complex (MTBC) was initially based on an IS6110 restriction fragment length polymorphism (RFLP) analysis (Almeida et al., 2019). Owing to the complexity of the RFLP method and the theoretical and practical limitations, new alternative molecular epidemiology tools have been developed to distinguish relapse or re-infection, assess risk factors associated with transmission, track infection transmission dynamics, and detect suspected outbreaks (Kone et al., 2020 Smith et al., 2017). These new methods are polymerase chain reaction (PCR)-based with simpler, more reliable techniques introduced as TB molecular typing tools such as spoligotyping and mycobacterial interspersed repetitive units–variable-number of tandem repeats (MIRU-VNTR) assays (Kamerbeek et al., 1997 Rozo-Anaya and Ribón, 2010; Supply et al., 2006). Spoligotyping, referred to as spacer oligonucleotide typing, is a widely used PCR-based method for genotyping MTBC organisms (Huard et al., 2003 Zeng et al., 2016). It detects the presence or absence of unique spacer sequences present in the direct repeat (DR) genomic region of MTBC by a reverse line blot hybridization approach (Honisch et al., 2010). MIRU-VNTR is a more recent molecular MTB strain-typing method developed and introduced into the platform of molecular typing tools, which uses the variations observed from strain to strain in 12 to 24 independent minisatellite-like loci scattered throughout the MTB genome (Allix-Béguec et al., 2008b). MIRU-VNTR typing have emerged as valuable markers for genotyping of MTBC (Allix et al., 2004 Allix-Béguec et al., 2008a; Alonso-Rodríguez et al., 2008; Oelemann et al., 2007; Shamputa et al., 2006). Furthermore, the combination of spoligotyping and MIRU-VNTR more accurately characterizes the genetic diversity and molecular epidemiology of MTBC strains circulating in the clinical isolates of a given population, estimates recent transmission of TB, and possibly detects active transmission of TB (Shi et al., 2018 van Deutekom et al., 2005).

To date, in Mali, all molecular epidemiology studies have only used spoligotyping (Diarra et al., 2018 Togo et al., 2017; Traore et al., 2012), resulting in a poor epidemiological characterization of TB cases. Therefore, this study sought to use both molecular methods —spolygotyping and MIRU-VNTR—to characterize the diversity of MTB genotypes of clinical isolates from patients admitted in multiple municipalities in the capital city of Mali, Bamako, where a third of all TB cases are reported. The findings from this study contribute to a better understanding of TB hot spots and the phylogenetic diversity of MTBC circulating strains in Bamako.

Methods

Study Setting, Population, Sample Size Determination, and Ethical Consideration

A cross-sectional study was conducted from January 2017 to December 2019 at the University Clinical Research Center (UCRC) of the University of Sciences, Techniques and Technologies of Bamako (USTTB), Mali. Confirmed cases of patients with TB who agreed to participate signed a consent form before enrolment. All participants were enrolled in the study from the 6 referral health centers (RHCs) in Bamako, RHC I, II, III, IV, V, and VI, where TB diagnosis and treatment are performed routinely. Bamako is the capital city of Mali, with an estimated 2.7 million inhabitants who represent one-tenth of the Malian population (Atlas of populations and countries of the world, 2020). According to the Mali

National TB/HIV Program, Bamako is where a third of TB infection takes place and is considered to be the highest transmission area (National TB, HIV and Hepatitis program, 2020). Participants in the study included males and females aged more than or equal to 18 years with confirmed cases of active pulmonary TB.

The sample size was calculated in accordance with a previous study conducted in Bamako (Traore et al., 2012). In general, there is low prevalence of mycobacterial species circulating in Mali; however, the lineage 4 (T1 family and LAM) is the most dominant, followed by lineage 6 (*M. africanum*). We assumed that the 2 predominant strains are likely to form clusters; therefore, our sample calculation was based on *M. africanum* prevalence (the least frequent of the 2 predominant strains) estimated to be 28% (Traore et al., 2012). Epi Info 3.5.3 software was used to calculate the study sample size. Expecting the prevalence of *M. africanum* greater or equal to 20%, a power of 80%, and α at 0.05, with a number of 2090 new TB cases reported in Bamako in 2017, we retained a total of 221 participants as our sample size.

The research protocol and the consent form were first approved by the ethical committee of the Faculty of Medicine and Odontostomatology (FMOS) of the University of Sciences, Techniques and Technologies of Bamako (USTTB) before any research activity (ethical reference number: N°2018/97/CE/FMOS).

Sputum Sample Collection, Mycobacterial Sputum Culture, and DNA Extraction

Sputum samples were collected from potentially eligible patients and treated according to the standard operating procedure. Briefly, digestion and decontamination methods were applied using sodium hydroxide and N-acetyl-L-cysteine solutions, then the mixture was concentrated through centrifugation at 3000 × g. Pellets obtained from the centrifugation were used to inoculate liquid and solid TB culture media. Smears were also prepared with the processed sputum pellets for microscopic visualization of acid-fast bacillus (AFB). (Traore et al., 2012). DNA was extracted from the pure isolates of MTB for molecular characterizations (Jafarian et al., 2010).

Spoligotyping

A commercially available kit was used to perform the spacer oligonucleotide typing and the manufacturer's instructions were followed (Isogen Bioscience BV, Maarssen, The Netherlands). Briefly, an implication of the DR locus was done with primers Dra and Drb using PCR, and then, the amplicons were hybridized to a set of 43 immobilized oligonucleotides covalently bound to a membrane. The hybridized PCR products were incubated with streptavidin peroxidase conjugate and the results were detected by chemiluminescence system (ECL detection). Strain comparisons were made using the SPOTCLUST (SpolDB3-based) database. Corresponding shared spoligotypes were further defined using the SITVIT database (Institut Pasteur de la Guadeloupe, Abymes, Guadeloupe). (Kamerbeek et al., 1997) H37Rv and BCG strains were used as internal controls.

Variable-Number Tandem Repeats of Mycobacterial Interspersed Repetitive Units (MIRU-VNTR) Test

The GenoScreen MIRU-VNTR typing kit, using which the 24 markers are amplified from purified DNA or crude extract using 6 quadruplex PCR and fluorescent primers specific for the flanking regions of the targeted loci, was used in this study as previously described (Variable-Number Tandem Repeats of Mycobacterial Interspersed Repetitive Units, 2020).

Raw data (.fsa files) were analyzed using GeneMapper v5.0 software. Proprietary GeneMapper modules and panels were used to match fragment sizes to each MIRU-VNTR locus repeat number. In case of failure or double alleles, the analysis was repeated once, except if more than 4 alleles were missing. Strain identification was performed using the MIRU-VNTRplus database, following a strategy described previously (Allix-Béguec et al., 2008b). Briefly, VNTR patterns were first matched against the MIRU-VNTRplus database, using a default stringent distance cutoff of 0.17, which corresponds to a tolerance of, at most, 4 locus differences. In cases of no match detected after the initial best-match analysis, a tree-based identification was used. The phylogenetic tree was calculated and drawn by using the neighbor-joining algorithm, using the MIRU-VNTRplus database reference strains (BCG strain). The most straightforward categorical distance was used by default. This distance simply scores the number of markers with a different allele divided by the total number of markers used. Missing data were ignored to accommodate the incorporation of strains with incomplete data. However, to avoid the overinterpretation of results, sample identification was usually not performed if more than 1 marker is missing. BCG strain was used as internal controls.

Statistical Analysis

Data were encrypted and analyzed using SPSS version 25 for Windows (IBM SPSS Statistics 25). Frequencies were calculated for all descriptive variables. Fisher exact test or chi-square test was used for the comparison of the frequencies. Variables with a P value <.05 were considered statistically significant. Geographic origin and strain diversity profiles with spoligotyping families or MIRU-VNTR were made using QGIS software version 3.10.

Results

Clinical and Sociodemographic Characteristics of Participants

A total of 245 participants from 6 respective RHCs (I, II, III, IV, V, and VI) were enrolled in the study. Of the 245 participants, n = 16 (6.5%) were patients co-infected with HIV and n = 9 (3.7%) were multidrug resistant TB (MDR-TB) cases (Table 1). A small number were TB cases being retreated (n = 34, 13.9%), with the remaining being new cases (n = 211; 86.1%). Overall, most participants were male (n = 171, 69.8%) with a sex-ratio of 2.3 with participants' mean age of 33 ± 11.7 years (range 18–76). The most predomi nant isolates were from the referral health centers (RHCs) V and VI (with n = 37, 20.10% and n = 82, 44.56%, respectively) (Table 2 and Figure 1).

Identification and Genotyping of M. Tuberculosis Using Spoligotyping

All 245 isolates were successfully classified by the spoligotyping method, in which the largest spoligotype family observed was LAM-10, which accounted for 82 isolates (33.47%). The next most common family was the T1 family with 78 isolates (31.84%), followed by the *M. africanum* family with 39 isolates (15.92%). A few isolates belonged to Beijing (n = 6, 2.45%), Haarlem-3 (n = 5, 2.04%), *M. bovis* (n = 5, 2.04%), Family-33 (n = 12, 4.9%), LAM-7 (n = 4, 1.63%), LAM-3 (n = 3, 1.22%) LAM-9 (n = 3, 1.22%), X3 (n = 4, 1.63%), EAI5 (East African-Indian-5) (n = 3, 1.22%) and Family-34 (n = 1, 0.41%) (Table S1).

M. tuberculosis Strain Identification and Genotyping Using MIRU-VNTR and Spoligotyping

Of the 245 isolates that were genotyped by MIRU-VNTR, 61 isolates were found to be indeterminate (Table S2). As a result, only 184 were fully analyzed, which revealed that Cameroon, Ghana, LAM, West African-2, and H37Rv strains of MTB were present in 5 of the 6 referrals health centers. The Cameroon type was highly represented in RHC V and VI with respectively 50 (60.2%) and 12 (14.5%), whereas the Ghana types was mostly found in the RHC III with 10 (37.0%). The West African 2 strain was found to be predominant in RHC V with 10 (45.5%) and the LAM strains with 3 (37.5%) in RHC V. The H37Rv type was mostly identified in RHC II, IV, and VI with 2 (25%) (Table 2).

Notably, 184 strains were grouped into 8 major clusters: 45.1% (83 isolates) of Cameroon, 14.7% (27 isolates) of Ghana, 12% (22 isolates) of West African 2, 7.1% (13 isolates) of Haarlem, 4.3% (8 isolates) of H37Rv(t) and LAM, 3.8% (7 isolates) of Beijing, and 2.7% (5 isolates) of Uganda I. Conversely, 3 isolates (1.6%) presented a single strain with MIRU-VNTR assay (Table 3). The distribution of study isolates into various clusters using MIRU-24 and spoligotyping databases is shown in Table 3. The majority of LAM-10, T1 family, identified by spoligotyping solely were reclassified as Cameroon and Ghana by the combination of spoligotyping and MIRU-VNTR. Most of H37Rv strains identified by the MIRU technique were classified as T1 family. The majority of *M. africanum* family remained the same with both techniques (Table 3). Five isolates of Family-33 for spoligotyping test were classified by MIRU as being Cameroon, Ghana, West African 2, Haarlem, and H37Rv. Overall, the clusters rate was 68.64%. In contrast, the MDR-TB cases were mostly associated with the Ghana type in this study, where of the 9 isolates of MDR-TB, 7 were genotyped as Ghana. Statistical analysis showed a significant difference between the identified lineages from the referral health centers in Bamako, Mali ($P < 0.01$) in the exception of RHC IV with $P = 0.478$. (Table 4)

A few isolates did not provide any results for some loci. The most problematic loci were MIRU10 (VNTR 0960) and MIRU16 (VNTR 1644) and they failed for 1 allele in 36 cases. The failure to amplify MIRU10 (VNTR 0960) and MIRU16 (VNTR 1644) was associated with isolates belonging to the Cameroon family.

Transmission Links in Clusters

We further sought to identify potential links of transmission between the cases, using MIRU and spoligotyping similarities between the strains (a combination of all 24 loci) (Table 5).

The Cameroon clade revealed active ongoing community transmissibility with the highest numbers of clusters and cases in each cluster (SIT 61). A total of 6 clusters were found; of which, cluster 1 had 11 cases (Table 5). Two clusters involved *M. africanum* West African 2.

Phylogenetic Analysis

A neighbor-joining (NJ) tree of all the isolates is shown in Figure S1 in the supplementary material. We observed that the main families were well-distinguished with high diversity within and between families. There is a subdivision of MTBC species into 2 main groups. The first group includes the most virulent strains (Beijing, Cameroon, LAM and Delhi) and the second is composed of less virulent strains from West Africa (notably, Ghana and Haarlem).

Discussion

The results of this study showed a high diversity of *Mycobacterium tuberculosis* in Bamako, Mali. Based on these findings, the TB epidemic in Mali appears to be, in large part, a result of many imported cases, followed by intense local community transmissions. In 2000, WHO reported an incidence of TB in Mali of 77 cases per 100,000 people, which significantly decreased to 53 cases per 100,000 people in 2019 (World Health Organization, 2020). Despite the decreased incidence but high diversity in Mali, this study suggests that the nation should be concerned about the emergence of new strains with high potential of increased transmissibility and development of resistances, such as the Ghana and Cameroon strains, which are present in high numbers in the different RHCs in Bamako. This was evidenced by Senghor et al reporting that the Ghana genotype isolates appeared more likely to be associated with MDR-TB than other identified genotypes (Senghore et al., 2020).

The TB spoligotyping revealed that the most represented spoligotype family was LAM with 33.47% (82/245) of all isolates across the different RHCs of Bamako, followed by the T1 family with 31.84% (78/245), the *M. africanum* family with 15.92% (39/245), and Family-33 with 4.9% (12/245). The Beijing, Haarlem 3, *M. bovis*, LAM-7, LAM-3/LAM-9, X3, EAI5, and family-34 were least represented (<2%). In 2012, a cross-sectional study using spoligotyping method conducted in Bamako showed that the T1 family, *M. africanum* West African type 2, and the LAM-10 family were the most predominant strains (Traore et al., 2012). Another study reported the same 3 major families across Bamako, Mali, where T1 was 31.9%, LAM-10 was 15.3%, and *M. africanum* West African type 2 was 16.8%. Our study confirms these reports with spoligotyping, with a minor difference in that the LAM family was found to be the most prevalent in our analysis (Togo et al., 2017). This demonstrates that these MTB families remained predominant in this region of Bamako for many years and that a thorough investigation is needed to effectively identify the source of the infections and to shed light on the transmission links.

As predicted, solely spoligotyping had gaps that clearly delineated the circulating strains and the classification of the observed strains changed when the MIRU method was applied, highlighting the limitation of previous molecular epidemiology of TB in Mali. Thereby, the standard 24-locus MIRU-VNTR genotyping of MTBC improved the resolution power of tracking TB transmission and predicting different strains, lineages, and sublineages in a

specific area or community. Remarkably, in our study, the 24-locus MIRU-VNTR typing method identified 3 unique types and grouped them into 8 major clusters, which include the Cameroon, Ghana, West African 2, Haarleem, H37Rv, LAM, Beijing, and Uganda I. The Cameroon and Ghana strains were present in all referral health centers, with the exception of RHC IV, and this can be explained by the sample size of this health center (with only 6 of 184). In contrast, the H37Rv (t) was found in 5 of the 6 RHCs (Table 2). A previous study reported that the dominant lineage was the Euro-American lineage, and the Cameroon genotype was the most prevalent genotype, followed by the Ghana genotype (Senghore et al., 2020), which was confirmed by our current study. The genotype Ghana was associated with 77.78% of MDR-TB appearing to be a highly representative strain in 2 of the 6 RHCs, especially RHCIII with 37%. The emergence of this strain is worrisome and should be followed and managed appropriately. Overall, the high clustering rate of 68.64% in this study is indicative of local transmission across the RHCs. High mycobacterial diversity with many European and Asian origins suggests continuous importation of cases. The Cameroon(s) cluster was the most predominant in this study.

To identify and accurately classify the mycobacterial species and better establish the molecular epidemiology profile of TB in Mali, we combined spoligotyping and MIRU methods because both methods are complementary for TB strain-typing. Spoligotyping and MIRU-VNTR technologies differentially identified the MTB strains, the majority of LAM-10 and T1 family identified by spoligotyping were classified as Cameroon(s) and Ghana(s) by MIRU-VNTR, respectively. The H37Rv strains in MIRU were also classified as T1 family. However, the majority of the *M. Africanum* family was classified the same by spoligotyping and MIRU-VNTR technologies (Table 6). Family-33, identified by spoligotyping, was classified as Cameroon(s), Ghana(s), West African 2(s), Haarlem(s), and H37Rv by MIRU, showing that MIRU provided better resolution in classifying MTBC at sublineage levels. Five distinct lineages were identified in this study (lineage 1, 2, 3, 4, and 6), each of them grouping together various MTB strains and sublineages (Table 3). Lineage 4 represented the most diverse strains and sublineages, followed by lineage 6. In 2016, Winglee et al found similar patterns of diverse lineages and demonstrated that they are involved in drug resistance through similar mechanisms (Winglee et al., 2016). Togo et al also showed that these 2 lineages have been the leading sources of TB infection in different parts of Bamako for a decade (Togo et al., 2017). Statistically significant differences were observed between the identified lineages across the RHCs, confirming distinct and diverse MTB strains in different parts of the RHCs.

The hierarchical clustering of the observed MTB strains is illustrated in a dendrogram (Figure S1), where the genetic relatedness of most predominant families are shown, based on the neighbor-joining (NJ) tree constructed using the 24-loci MIRU-VNTR database. This phylogenetic analysis clearly demonstrates that MTBC isolates from the 6 RHCs are represented by several distinct groups, mainly 2 distinct groups, each comprising various subgroups. The main Cameroon clusters found in Bamako showed similarity in all 24 loci, meaning that the cases are likely from the same chain of transmission. Although our investigation did not establish a formal link, it can be explained, in part, by the wide use of public transportation by many infected patients and the lack of precautions to prevent TB. Other places of high transmission are potentially common households and use of communal

public toilets. Social and cultural norms in Mali do not encourage the isolation of patients even with known TB infection. Furthermore, the hypervirulence and hyper-transmissibility of the Cameroon clade are considered as contributing factors.

Limitation

This is one of few studies to use spoligotyping and the MIRU-VNTR techniques together to genotype the different MTBC and the first of such studies in Mali. Despite these encouraging results, the limitations of this study include difficulties encountered in processing MIRU-VNTR samples because of the limited amount of DNA in some cases. The results of the study are not generalizable to Mali, given that all participants were from Bamako. Nevertheless, this study is the first to use the most up-to-date tools to determine the molecular profile of TB in Bamako with greater accuracy. This study should guide future epidemiological characterization and be used to target the transmission of both imported and community strains.

Conclusion

By combining spoligotyping and MIRU-VNTRs 24-loci technologies, a more comprehensive view of MTBC distribution in Bamako, Mali was achieved. The predominant strain circulating in Bamako was Cameroon(s). The MIRU-VNTR technology achieves a high level of biodiversity discrimination and provides efficient subclustering to considerably reduce the number of potential epidemiological links. The geographic variability of MTBC strains may allow health authorities and researchers to track the global spread of TB. Indeed, the elimination and eradication of this disease will only be possible if prevention efforts are more effectively targeted at hot spots where the disease is widespread. Advanced molecular techniques allow for resistant strains to be rapidly identified and break the chain of transmission. These results should be used as proof of concept to initiate a national ongoing evaluation of MTBC genetic diversity in Mali.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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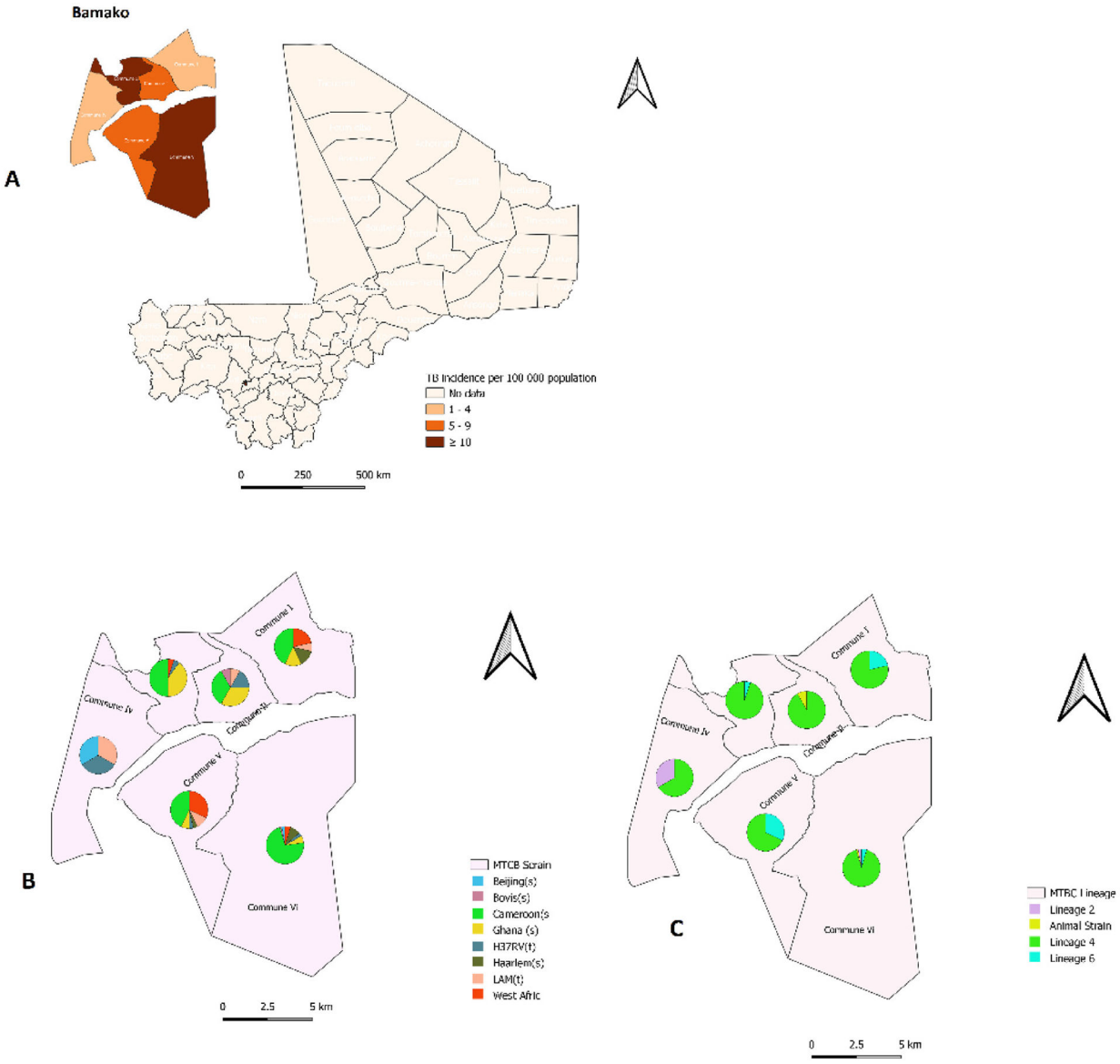

Figure 1.

Structure of the urban district of Bamako with the distribution of lineages based on the combination of MIRU and spoligityping methods. (a) Describes the distribution of population density per tuberculosis and the localization of the health center; (b) describes the distribution of the most population by strain; and (c) describes the distribution of TB cases recruited by lineage. MIRU, mycobacterial interspersed repetitive unit.

Table 1

Demographic and clinical characteristics of study participants.

Characteristics (n = 245)		All participants n (%)	Modern lineages[a] (N = 193) n (%)	Ancestral lineages[b] (N = 47) n (%)	Animal strains[c] (N = 5) (%)	*P* value
Age (years)	18–30	128 (52.2)	106 (54.9)	21 (44.7)	1 (20.0)	0.394
	31–45	83 (33.9)	62 (32.1)	17 (36.2)	4 (80.0)	0.751
	46–60	25 (10.2)	17 (8.8)	8 (17.0)	0 (0)	0.555
	> 60	9 (3.7)	8 (4.2)	1 (2.1)	0 (0)	0.923
Sex	Male	171 (69.8)	139 (72.0)	28 (59.6)	4 (80.0)	0.193
	Female	74 (30.2)	54 (28.0)	19 (40.4)	1 (20.0)	0.319
HIV status	Positive	16 (6.5)	11 (5.7)	4 (8.5)	1 (20.0)	0.850
	Negative	196 (80.0)	157 (81.4)	36 (76.6)	3 (60.0)	0.513
	Not done	33 (13.5)	25 (12.9)	7 (14.9)	1 (20.0)	0.892
Sputum microscopy	Grade 1 (Few AFB)	30 (12.2)	18 (9.3)	11 (23.4)	1 (20.0)	0.305
	Grade 2 (Moderate AFB)	35 (14.3)	22 (11.4)	10 (21.3)	3 (60.0)	0.455
	Grade 3 (Many AFB)	180 (73.5)	153 (79.3)	26 (55.3)	1 (20.0)	0.008
Patient TB status	Newly Diagnosed	211 (86.1)	165 (85.5)	41 (87.2)	5 (100.0)	0.780
	Retreatment	34 (13.9)	28 (14.5)	6 (12.8)	0 (0)	0.915
Drug susceptibility profile	Pan Sensitive	185 (75.5)	143 (72.6)	38 (80.8)	4 (80)	0.305
	Other resistance	51 (20.8)	43 (22.3)	7 (14.9)	1 (20)	0.660
	MDR	9 (3.7)	7 (3.6)	2 (4.3)	0 (0)	0.965

Abbreviations: AFB, acid-fast bacillus; MDR, multidrug resistant; TB, tuberculosis.

[a]Modern lineage: L2, L3, L4.

[b]Ancestral lineage: L1, L5/L6.

[c]Animal strains = *M. bovis*.

Table 2

Distribution of TB strains and their classification into various TB lineages across the 6 referral health centers (RHCs) in Bamako, based on the MIRU-24 identification method.

Strain (N = 184	TB Lineage	Effective n (%)	RHC-I N = 21 n (%)	RHC-II N = 16 n (%)	RHC-III N = 22 n (%)	RHC-IV N = 6 n (%)	RHC-V N = 37 n (%)	RHC-VI N = 82 n (%)
Bovis	Animal strain	3 (1.6)	0 (0.0)	1 (33.3	0 (0.0)	0 (0.0)	1 (33.3)	1 (33.3)
EAI	Lineage 1	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (33.3)
Beijing	Lineage 2	7 (3.8)	0 (0.0)	1 (14.3)	0 (0.0)	2 (28.6)	0 (0.0)	4 (57.1)
Delhi/CAS	Lineage 3	3 (1.6)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)
Cameroon	Lineage 4	83 (45.1)	7 (8.4)	4 (4.7)	10 (12.0)	0 (0.0)	12 (14.5)	50 (60.2)
Ghana		27 (14.7)	5 (18.5)	5 (18.5)	10 (37.0)	0 (0.0)	2 (7.4)	5 (18.5)
H37Rv		8 (4.3)	0 (0.0)	2 (25.0)	1 (12.5)	2 (25.0)	1 (12.5)	2 (25.0)
Haarlem		13 (7.1)	2 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)	2 (15.4)	9 (69.2)
LAM		8 (4.3)	1 (12.5)	1 (12.5)	0 (0.0)	2 (25.0)	3 (37.5)	1 (12.5)
S		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
TUR		1 (0.5)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Uganda I		5 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (80.0)	1 (20.0)
Uganda II		1 (0.5)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
West African 2	Lineage 6	22 (12.0)	3 (13.6)	1 (4.5)	1 (4.5)	0 (0.0)	10 (45.5)	7 (31.8)

Abbreviations: CAS, Central Asian Strain; EAI, East African-Indian; LAM, Latin American Mediterranean; MIRU, mycobacterial interspersed repetitive unit; TB, tuberculosis; TUR, Turkish; RHC, Referral Health Center.

Table 3

TB isolates classification based on spoligotyping solely and in combination with 24-MIRU-VNTR

Strain assignment using spoligotyping solely N = 184	Strain assignments using 24 MIRU-VNTR loci and spoligotyping N = 184
LAM-10 (n = 64)	Cameroon (n = 83)
T1 (n = 10)	
X3 (n = 1)	
Family 33 (n = 1)	
X5 (n = 1)	
M. africanum (n = 2)	
X7 (n = 1)	
LAM-10 (n = 3)	
T1 (n = 18)	Ghana (n = 27)
Harlem 3 (n = 1)	
Family 33 (n = 1)	
T1 (n = 7)	
M. africanum (n = 14)	West African 2 (n = 22)
T1 (n = 1)	
Family 33 (n = 1)	
M. africanum (n = 6)	
T1 (n = 5)	Haarlem (n = 13)
Haarlem 3 (n = 2)	
M. africanum (n = 1)	
Family 33 (n = 1)	
T1 (n = 3)	
Haarlem3 (n = 1)	
T1 (n = 6)	H37Rv (n = 8)
Family 33 (n = 1)	
X1 (n = 1)	
LAM-9 (n = 3)	LAM (n = 8)
LAM-7 (n = 3)	
LAM-3 (n = 1)	
T1 (n = 1)	
Beijing (n = 4)	Beijing (n = 7)
Beijing (n = 2)	
M. africanum (n = 2)	
T1 (n = 4)	Uganda I (n = 5)
Haarlem 3 (n = 1)	
M. bovis (n = 2)	Bovis (n = 3)
M. bovis (n = 1)	
T1 (n = 2)	Delhi/CAS (n = 3)
T1 (n = 1)	
EAI5 (n = 2)	EAI (n = 2)

Strain assignment using spoligotyping solely N = 184	Strain assignments using 24 MIRU-VNTR loci and spoligotyping N = 184
T1 (n = 1)	S (n = 1)
T1 (n = 1)	TUR (n = 1)
T1 (n = 1)	Uganda II (n = 1)

Abbreviations: CAS, Central Asian Strain; EAI, East African-Indian; LAM, Latin American Mediterranean; MIRU, mycobacterial interspersed repetitive unit; TB, tuberculosis; TUR, Turkish; VNTR, variable-number tandem repeat.

Table 4

Comparison of the distribution of the most represented lineage 4 vs to the nonlineages 4 (animal strain, lineage 1, lineage 2, lineage 3, lineage 6) across the 6 referral health centers in Bamako, Mali.

Referral health centers (RHC)	Lineage 4	Nonlineage 4[a]	*P* value
RHC I	16 (76.2)	5 (23.8)	0.037
RHC II	13 (81.3)	3 (18.7)	0.039
RHC III	21 (95.5)	1 (4.54)	0.002
RHC IV	4 (66.7)	2 (33.3)	0.478
RHC V	25 (67.6)	12 (32.4)	0.046
RHC VI	68 (82.9)	14 (17.1)	0.0001

Table 5

gotyping within the «Cameroon» (CAM) and West African (2) clade.

D)	MIRU40 (0802)	MIRU10 (0960)	MIRU16 (1644)	Mtub21 (1955)	MIRU20 (2059)	QUB11b (2163)	ETR A (2165)	Mtub29 (2347)	Mtub30 (2401)	ETR B (2461)	MIRU23 (2531)	MIRU24 (2687)	MIRU26 (2996)	MIRU27 (3007)	Mtub34 (3171)	ETR E (3192)	Mtub39 (3690)	QUB26 (4052)	QUB4156 (4156)	MIRU39 (4348)
	3	2	3	3	1	5	4	4	2	2	5	1	5	3	3	3	3	5	2	2
	3	3	3	3	1	6	4	4	2	2	5	1	5	3	3	2	3	5	2	2
	3	3	3	3	1	6	4	4	2	2	5	1	5	3	3	3	3	5	2	2
	3	3	3	3	1	4	4	4	2	2	5	1	5	3	3	3	4	6	2	2
	4	3	2	3	1	3	3	4	2	2	5	1	5	3	3	3	1	5	2	2
	3	3	3	3	1	6	4	4	2	2	5	1	5	3	3	3	4	5	2	2
	3	3	3	3	1	6	4	4	2	2	5	1	5	3	3	3	3	4	2	2
	3	3	3	3	1	5	4	4	2	2	5	1	5	3	3	3	3	5	2	2

itive unit; SIT, Spoligo-international type; VNTR, variable-number tandem repeat.