Diversity of Mycobacterium *tuberculosis* strains in Nairobi, Kenya.

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SUMMARY

Setting: Tuberculosis (TB) patients attending 16 public health facilities in Nairobi, Kenya.

Objective: To determine the *Mycobacterium tuberculosis (M.tuberculosis)* strain families circulating in Nairobi, Kenya.

Methods: Sputum specimens from consecutive new and previously treated smear positive pulmonary TB patients were collected between February and August 2010 and cultured on Lowenstein-Jensen media. Spoligotyping was done on DNA extracted from the first isolate of each patient. The international spoligotype data base (SpolDB4) was used to group isolates into strain families.

Results: Fourty seven different strain families were identified from 536 isolates. The principal groups were; CAS1_KILI 96/536 (17%), T1 69/536 (12%), Beijing 65/536 (12%), LAM9 46/536 (9%), LAM3 & S/Conversant 37/536 (7%), LAM11_ZWE 26/536 (5%), CAS1_DELHI 24/536 (4%) and T2 24/536 (4%). Others identified and are found in the SpolDB4 were 113/536 (21%). A possible new *M.tuberculosis* strain family was identified with 21/536 (4%) isolates which was designated as *Nairobi subtype*. Others identified not previously included in the SpolDB4 accounted for 15/536 (3%).

Conclusion: We found a diverse array of *M.tuberculosis* strain families which could be indicative of a cosmopolitant polulation with frequent migration that may suggest that the dorminant strain families may have been present in the population for an extended period of time or on going transmision of closely related strains families. The emergence of the Beijing strains poses a serious threat to TB control due to its high virulence and frequent association with multidrug resistance. We therefore call for strenghthening efforts on early case finding through enhanced public health education campains and provision of accessible diagnostic services with enhanced treatment compliance.

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Introduction

Tuberculosis (TB) disease is an important public health problem all over the world including Kenya despite the availability of anti-tuberculosis drugs. The causative agent, *Mycobacterium tuberculosis* (*M. tuberculosis*) has continued to claim its victims throughout much of the known human history and is still one of the most significant infectious agents that cause death, ranked seventh globally among causes of death.[1] TB accounts for 2.5% of the global burden of disease and in 2007, the World Health Organization (WHO) estimated 9.27 million TB cases to have occured.[2,3] Low-income people living in dense urban communities with deficient housing conditions, have a high probability of becoming infected, developing active disease and dying from TB. Kenya is among the 22 high burden countries that together contribute 80% of the global TB cases.[3] In Kenya TB cases have been increasing from 11,635 cases in 1990 to 116,000 cases in 2007. In Nairobi alone, TB cases have increased from 1,048 in 1990 to 18,906 cases in 2007, which represents almost 18-fold increase in the last 17 years.[4] The emergence of HIV has greatly contributed to the increase in the number of TB cases in Nairobi and Kenya as a country. The HIV prevalence among the general population in Nairobi is 9%. This is higher than that for the country, which stands at 7.4%. [5]

Early case finding and treatment initiation are the most important measures to prevent the spread of TB in a community. Prolonged exposure to an infectious source enhances the chance of transmission and hence direct and close contact with a TB patient is a main cause of infection. However, in high TB prevalence areas the transmission routes are less clear.

Deoxyribonucleic acid (DNA) fingerprinting has become a powerful epidemiologic tool to understand the strain dynamics of *M. tuberculosis*. Since the past 2 decades, a variety of genetic makers were identified and used to study the molecular epidemiology and spread of *M. tuberculosis.*[6] Based on specific genetic markers, various *M. tuberculosis* genotype families have been identified, such as the Haarlem family,[6] the Beijing family,[7] Family 11,[8] the Manila family,[9] the Delhi family,[10] the Cameroon family,[11] and the Latin American Mediterranean (LAM) family.[12,13,14,15] These techniques are often not freely available in resource poor countries with a high burden of TB and therefore little is known of the population structure of the circulating strains in these areas.

In many molecular epidemiological studies, it has been found that only a minority of the epidemiological links between TB cases disclosed by DNA fingerprinting, are also found by conventional contact tracing based on interviews. [16,17,18,19,20] This suggests that a large part of the TB transmission takes place through casual contacts in public places, such as bars, discothèques, public transportation, or other crowded settings. These contacts will generally not be found by interviews. In a recent study done in South Africa, it was found that only 46 % of 313 TB patients had a matching fingerprint with an isolate of another member of the household they were living in. The proportion of transmission in the community that took place in the household was found to be only 19 %. The authors suggested that in this area, and presumably, also in other high-incidence settings, TB transmission mainly occurs outside the households. [21] This may also be the case in the highly populated areas in Nairobi. For proper TB management, it is important to get an insight into the strain types of *M. tuberculosis* that fuel the TB epidemic in Nairobi. A previous study conducted on

a small sample size (n=73) showed that the drug resistant Beijing/W genotype was present in Kenya.[22] However, no large population based study has been done yet to describe the molecular epidemiology of *M. tuberculosis* strains that circulate in Nairobi with a population of 3.1 million people according to the 2009 national population census. In this study we hypothesize that there will be numerous different strain types of *M. tuberculosis* that drive the TB epidemic and that this will be disproportionate in different health districts within Nairobi. Our objective therefore was to determine the *M. tuberculosis* strain types circulating in Nairobi and further understand strain dynamics in different health districts within Nairobi city.

Materials and Methods

Setting: The study was conducted in Nairobi, the capital city of Kenya. The population of Nairobi is steadly growing due to rural–urban migration and immigration from unstable countries e.g Somalia and Sudan. A significant proportion of the residents of Nairobi belong to the middle or low social economic class with high population densities in certain areas. The total area of Nairobi is 696 sq km with about 60% of the people living in less than 5% of this area in overcrowded informal settlements in the form of shelters. Nairobi has the highest TB case load compared to other regions in Kenya. In 2007 there were 18,902 (684/100,000) of all forms of TB cases and 6,634 (242/100,000) of the new smear positive TB cases.

There are 8 health districts in Nairobi divided based on the geographical location and population sizes for operational issues. All the 8 districts were selected for the study. The study was cross sectional and to get the number of patients per district the required sample size was divided proportionately to the 8 districts depending on the number of smear positive TB patients diagnosed by each district in 2007. A list of all public diagnostic centers in each district was compiled and ranked according the number of new smear positive TB cases diagnosed in 2007. The intake period was between February and August 2010 i.e at 6 months maximum duration in order to minimise operational costs. The diagnostic facility in each of the health districts with the highest number of patients diagnosed in a quarter of a year was selected. This approach was used because the facilities selected would capture most of the TB patients in that particular health district and reach the required sample size within the intake period. If the calculated number of patients in the first facility was not adequate for the particular district, then additional patients were recruited from the next nearest dignostic facility within the district. Consecutive eligible patients (both new and previously treated) diagnosed and/or registered for treatment at each of the selected diagnostic sites during the intake period and who gave consent were enrolled for the study .

Specimen collection and transport

At the peripheral laboratory, the standard Acid –fast (AFB) direct smear microscopy using Zeihn–Neelsen (ZN) staining was done on the initial sputum to confirm TB diagnosis of suspected patients. A second sputum specimen was then collected before start of treatment and then transported to the Central reference Laboratory (CRL) on the same day for culture. The central reference laboratory is located within the Centre for Respiratory Diseases Research, Kenya Medical Research Institute (CRDR– KEMRI) at Kenyatta National Hospital. Sputum specimens were first decontaminated using 4% sodium hydroxide to eliminate the associated commensal flora and then cultured on egg based solid media, Lowenstein–Jensen (LJ). The slopes were examined weekly for any

visible growth. A positive culture of *M. tuberculosis* confirmed the diagnosis of active TB disease.

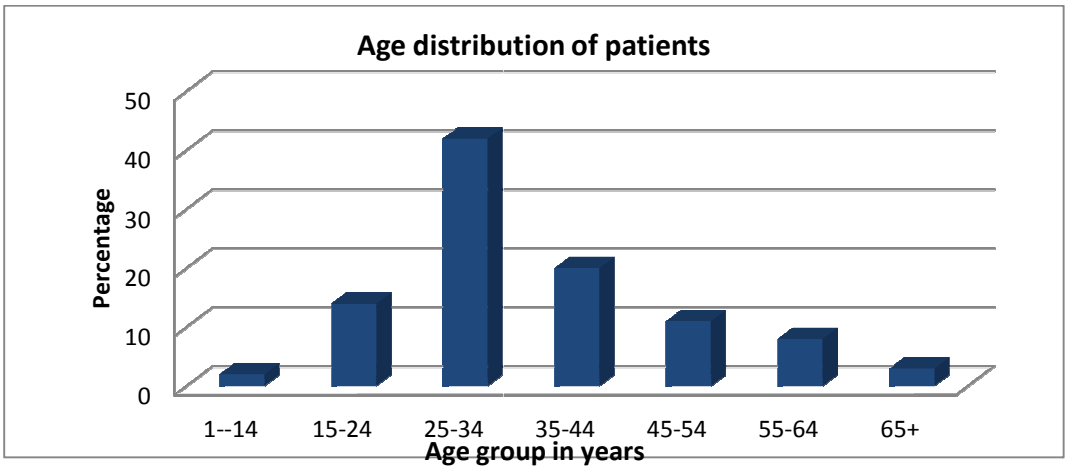
Genotyping of M. tuberculosis by Spoligotyping.

A scrap from the growth in LJ media was added to 200µl of distilled water and boiled for 20 minutes to inactivate the bacteria and to release a crude DNA template for Polymerase Chain Reaction (PCR) amplification. Currently no laboratory does spoligotyping in Kenya. Spoligotyping was done at the University of Stellenbosch, South Africa using the internationally standardized method. Spoligotype blots were visually inspected by two independent investigators and then the spoligotypes were grouped into strain families according to the international spoligotype database (SpolD4B).

Results

Five hundred and thirty six positive cultures of *M. tuberculosis* from pulmonary smear positive TB patients were available for analysis of whom 316 were new cases and 220 were previously treated. Of the 536 patients whose *Mycobacterium tuberculosis* positive cultures were analyzed, 339 (63%) were males and 197 (37%) were females. Information about age was available for 491 patients and 373 (76%) were between ages 15 to 44 years. The mean age was 31years with the minimum and maximum age of 14 and 84 years respectively (Figure 1). Status of HIV was known for 235 patients who were tested for HIV after consenting and 88 (37%) were HIV positive. Of the HIV positive 71 (81%) were between ages 15–44 years.

Figure 1: Age distribution of the patients.



A total of 536 isolates were Spoligotyped and could be grouped into 35 strain families previously identified and 12 families which were not in the international Spoligotype database (Table 1). The five major strain families were; CAS1_KILI 96/536 (17%), T1 69/536 (12%), Beijing 65/536 (12%), LAM9 46/536 (9%) and LAM3 & S/Conversant 37/536 (7%). Other strain families in the international spoligotype data base were 113/526 (48%). The distribution of these five main strain families in the 8 health districts within Nairobi is shown in Figure 2. The results show that in six health districts CAS1_KILL was the predominant strain type just like the entire Nairobi while in

two health districts T1 was the predominant strain type. One strain family with 21/536 (4%) isolates that was not in the international Spoligotype data base with absence of spacers 4, 10, 21 and 33–36 was designated as *Nairobi subtype*. The other eleven train types with 15/536 (3%) isolates that were not in the international Spoligotype data base were referred to as orphan types.

Table 2a: Main spoligotypes of *Mycobacterium tuberculosis* strain families in Nairobi.

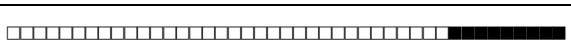
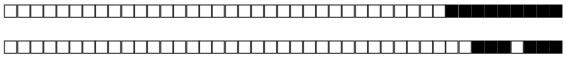
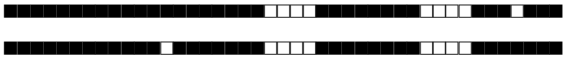
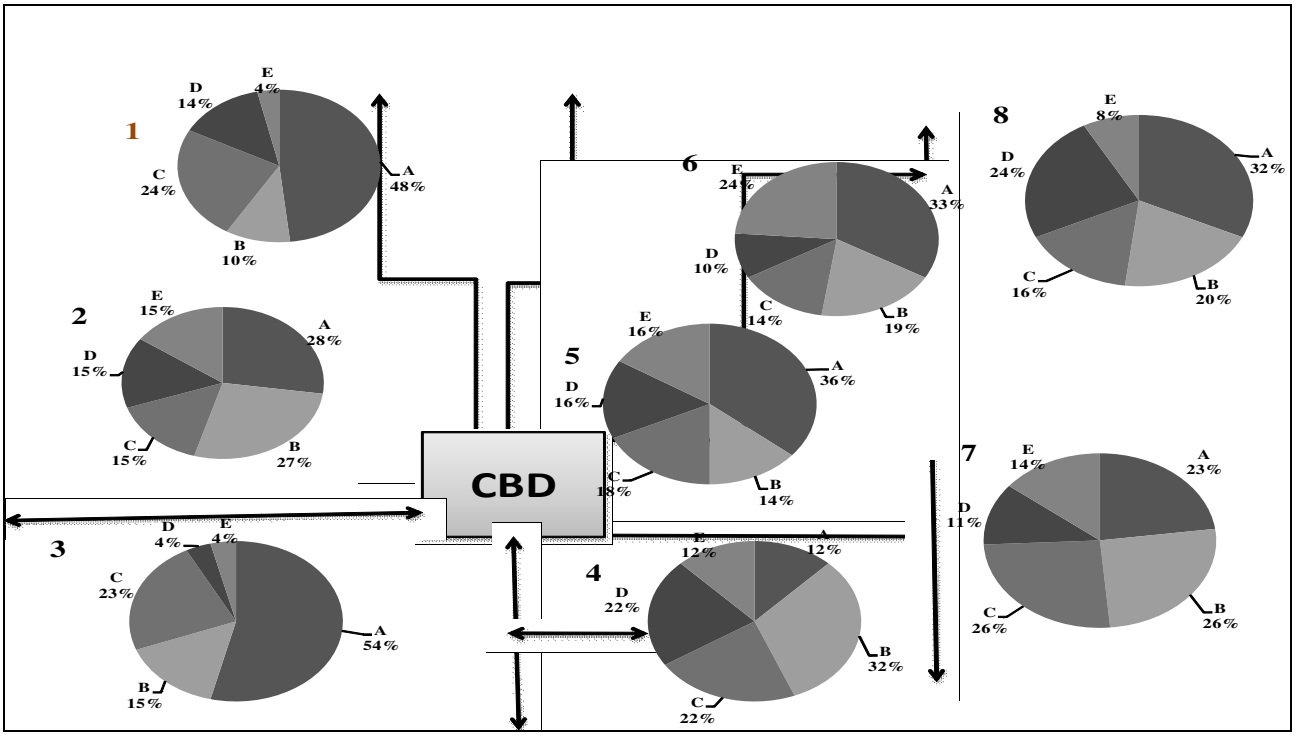
Family	IntType	Sub-group/Ffamily	Spoligotype pattern	No of Isolates
Beijing	1	BEIJING		65
	406	BEIJING-LIKE		1
Central -Asian (CAS)	26	CAS1_DELHI		24
	21	CAS1_KILI		96
	288	CAS2		4
	22	CAS (Others)		5
East African-Indian (EAI)	924	EAI5		5
	8	EAI5 or EAI3		6
		EAI (Others)		5
Haarlem (H)	218	H1		10
	50	H3		10
	36	H3-T3		1
Latin America and Mediterranean (LAM)	20	LAM1		1
	60	LAM4		3
	93	LAM5		5
	64	LAM6		4
	42	LAM9		46
	61	LAM10_CAM		1
	59	LAM11_ZWE		26
	4	LAM3 and S /Convergent		37
		LAM (Others)		2
The T-Family	53	T1		69
	78	T1-T2		1
	420	T2		24
	73	T2-T3		1
	37	T3		8
	345	T3_ETH		12
		T (Others)		1
The X-Family	302	X1		1
	137	X2		10
	92	X3		1
	1470	X1-LAM9		1
Subtotal				486

Table 2b: Other spoligotypes of Mycobacterium *tuberculosis* strain families in Nairobi.

Family	IntType	Sub-group/Family	Spoligotype pattern	No.of Isolates
Others found in the SpolDB4	1778	U		11
	34	S		2
	54	MANU2		1
Others Not included in the SpolDB4		New Family Nairobi		21
		Orphan type 1		3
		Orphan type 2		1
		Orphan type 3		1
		Orphan type 4		1
		Orphan type 5		2
		Orphan type 6		1
		Orphan type 7		1
		Orphan type 8		1
		Orphan type 9		2
		Orphan type 10		1
		Orphan type 11		1
Subtotal				50

Figure 3: Distribution of the five main strain families within 8 Nairobi health districts.



LEGEND: 1 to 8 Indicate the different health districts:

1=Westlands, **2**=Dagoreti, **3**=Langata, **4**=Makadara, **5**=Starehe, **6**=Kamukunji, **7**=Embakasi, **8**=Kasarani :

A to E: Five major *M. tuberculosis strain families*:

A=CAS1_KILL, **B**= T1, **C**=BEIJING, **D**=LAM9, **E**= LAM3 & S/Conversant.

CBD= Central Business District. ⟷ Major Roads within Nairobi city

Discussion.

This is the first comprehensive study to describe the *M. tuberculosis* strain dynamics in Nairobi, Kenya. The results showed that the TB epidemic in Nairobi is driven by five main strain families namely; CAS1_KILI (17%), T1 (12%), Beijing (12%), LAM9 (9%) and LAM3 & S/Conversant (7%) which collectively contribute to 57% of the total TB epidemic. The same strain families were also identified in a small scale study conducted in 2004 in Nairobi. However the relative proportions were different; CAS1_KILI (24%), T1 (3%), Beijing (8%), LAM9 (11%) and LAM3 & S/Conversant (1%).[22] This confirms that the contribution of these strain families in fueling the TB epidemic has now increased. In the present study the overall proportional contribution of the epidemic in Nairobi by the Beijing family has increased from 8% to 12% while that of the T1 family has increased from 3% to 12%. The Central-Asian (CAS) or Delhi family was identified as the largest group of strains circulating in Nairobi in both studies. These increase in trend of *M. tuberculosis* strain dynamics in Nairobi may be a reflection of frequent migration and living in crowded areas with increased interaction of the people through efficient roads which connect to the city centre. Changes in social activities of the people in Nairobi may also have lead to these changes. This changes are is important to note because they indicate that the problem has increased with increased challenges such as diagnosing drug resistant *M. tuberculosis* strains so as to contain the spread of the deadly virulent strains especially Beijing family that has been associated with drug resistant TB.[22] Despite the fact that this study does not address the aspects of drug resistance, it is important to note that the Beijing family contributed a large proportion of strains and therefore may be a cause of concern for alarm if not closely monitored. Studies describing strain diversity have been performed in a few countries from Africa[14, 23]. Previously the CAS family has been shown to be endemic in Sudan and other sub-Saharan countries.[12] Recent molecular studies have shown that LAM10 was the largest strain family[24] in urban areas in Jos, Nigeria and LAM 11_ZWE the largest in Ndola, Zambia.[25] In Cape Town, Southern Africa the F11 family which is related to the LAM family was the largest strain type, being isolated in 21.4% of the patients.[8] In the present study the LAM family also contributed to a significant proportion of the isolates with LAM9 (9%) being the fourth largest group. This shows that the LAM family is well adapted and is responsible for TB disease in West, South and East African countries. However the two studies from West Africa did not identify the Beijing/W strain, which was the third most prevalent strain type, identified in our study and which is the most prominent drug resistant strain type in Cape Town, South Africa[26,27]. In the African region, 31% of all new TB cases in adults (aged 15-49 years) are attributable to HIV infection[27] and in the present study; we found the TB/HIV co infection rate to be 37%. HIV not

only does increase the risk of reactivating latent *M. tuberculosis* infection to TB disease but also increases the risk of rapid progression soon after *M.tuberculosis* infection or re-infection to active TB disease. In addition, the high burden of HIV associated TB cases in Nairobi may also expand *M. tuberculosis* transmission rates at the community level threatening the health and survival of HIV negative individuals as well.

We acknowledge that in the current study, Spoligotyping was used to type the strains and it is known that this method has less discriminatory power than other genotyping methods such as *RFLP IS6110* and therefore required additional genotyping. Secondly, we enrolled only smear positive patients, and given that smear negative and extra pulmonary TB patients account for a considerable number of TB cases in Nairobi. The study was conducted for a period about 6 months and this may give an incomplete picture of the strain diversity in Nairobi. However, smear positive patients are the most efficient sources of TB transmission and therefore represents the most common strains that are transmitted. Most of the patients diagnosed were young between the ages of 15-44 years (76%) with a mean of 31years. This could indicate on going active transmission of *M. tuberculosis* strains.

Conclusions

This study demonstrates that the TB cases in Nairobi are caused by a diverse array of *Mycobacterium tuberculosis* strain families that may be indicative of a cosmopolitant population with frequent migration. This may suggest that the dorminant genotypes may have been present in the population for an extended period of time or on going transmision of closely related strains. The emergence of the Beijing strains poses a serious threat to TB control due to its high virulence and frequent association with multidrug resistance . A new subtype strain family was identified which we have designated the subtype Nairobi. We call for strenghthening efforts on early case finding through enhanced public health education campains and provision of accessible diagnostic services with enhanced treatment compliance.

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