academicJournals

Vol. 10(35), pp. 1460-1466, 21 September, 2016
DOI: 10.5897/AJMR2016.8221
Article Number: 51CECB060595
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Molecular genotypes of *Mycobacterium tuberculosis* strains circulating in Dakar, Senegal

Awa Ba Diallo[1,5]*, Ossaga Gedeon Walbang[1], Makhtar Camara[1,5], Seynabou Lo[2], Abigail Ayorinde[3], Aliou Niang[4], Souleymane Mboup[1,5], Aissatou Gaye Diallo[1,5], Bouke Catherine de Jong[3,6] and Florian Gehre[3,6]

[1]Mycobacteriology Unit, Bacteriology Virology Laboratory at CHU Aristide Le Dantec, Dakar, Senegal.
[2]Faculty of Health Sciences, Gaston Berger University, Saint Louis Senegal.
[3]Medical Research Council Unit, Fajara, The Gambia.
[4]Pulmonology Clinic at CHU Fann Hospital, Dakar Senegal.
[5]Faculty of Medicine and Pharmacy, Department of Biological Sciences at Cheikh Anta Diop University Dakar, Senegal.
[6]Institut for Tropical Medicine, Antwerp, Belgium.

Received 19 June 2016; Accepted 19 August, 2016

Tuberculosis is a contagious infectious disease, in which epidemiologic monitoring by molecular approaches is a critical element of prevention and control. The population structure of the *Mycobacterium tuberculosis* complex (MTBc) in Senegal was last described in the 1970's using biochemical methods. In this present study, we applied molecular approaches to genotype *M. tuberculosis* isolates from active pulmonary tuberculosis patients who participated in a prospective cohort study between 2004 to 2006. Genetic characterization, using standard spoligotype analysis and Line Probe Assay for resistance to isoniazid and rifampicin, was applied after culture on egg based solid media. The prevalence of resistance to isoniazid was 1.0% and to rifampicin 0.0% among 203 isolates tested. Of the 203 isolates, spoligotype patterns present in the TB-insight database were identified in 178 (87.6%) while 25 (12.3%) showed patterns suggestive of mixed infection. The major spoligotypes identified were the Haarlem lineage (22%), followed by the T (19%), Beijing (12%), LAM (12%), and *M. africanum* West African 2 (10%). Patterns suggestive of mixed infections, such as the sole lack of spacers 33 and 34, suggested a combination of Euro-American *M. tuberculosis* and Beijing lineage, which were confirmed by polymerase chain reactions (PCRs) for lineage defining deletions in a subset of isolates. The population structure of the *M. tuberculosis* complex in Dakar reflects a predominance of Euro-American *M. tuberculosis* (Haarlem, T and LAM), with a decreased prevalence of *M. africanum* West African 2, compared with reports from the 1970's based on biochemical speciation, which reported prevalence of *M. africanum* around 20% in Dakar.

Key words: Tuberculosis, *Mycobacterium tuberculosis* complex, polymerase chain reactions (PCRs).

INTRODUCTION

Tuberculosis (TB) remains a major cause of illness and death worldwide, especially in Africa and Asia. In 2014, the National Tuberculosis Program (NTP) reported 13.647 new TB cases in Senegal, of which 7% were co-

infected with HIV. With a population of about 14 million inhabitants, TB incidence is estimated at 138 cases per 100,000 persons per year (all forms), and prevalence at 205 cases per 100,000 persons (WHO, 2015). Molecular genotyping tools for *Mycobacterium tuberculosis* have become valuable for TB diagnostics and investigations of disease transmission dynamics, outbreaks and phylogeny**.** They allow the distinction between "modern" MTBC lineages versus "ancient" lineages and their respective geographical distribution worldwide.

Although these DNA typing techniques in conjunction with classical epidemiology approaches greatly enhance the understanding of TB transmission dynamics and epidemiology, no such study has been recently performed in Senegal. The only report that characterized circulating strains in the country was conducted by Niang et al. (1999). The authors presented preliminary work on 69 isolates using spoligotyping as a molecular tool to have an overview of strains, circulating in Dakar. As the sample size was relatively small, we are presenting a more comprehensive analysis on the mycobacterial population structure in Dakar between 2004 to 2006 using, molecular genotyping.

MATERIALS AND METHODS

Patients

The study population consisted of consecutive consenting new TB patients older than 18 years, who were part of a cohort of the African Tuberculosis Vaccine (AFTBVAC) study between, 2004 and 2006. Patients were recruited based on TB index case and their potential contact within a family. All subjects were diagnosed with clinically and bacteriologically confirmed tuberculosis. After written informed consent, sputa were collected from patients by clinicians from the Pulmonology clinic at Fann Hospital, Dakar, Senegal. All enrolled patients were resident of Dakar or suburbs surrounding the capital. Demographic and clinical patient data including age, sex, HIV status and severity of disease on chest radiography were recorded. This study was approved by the Ethics Committee of the Ministry of Health, Senegal.

Laboratory analysis

Sputum examination and TB culture were done at the Bacteriology laboratory of Aristide Le Dantec University hospital in Dakar, Senegal. After staining with the Ziehl Neelsen method, sputa were decontaminated with N-acetyl cysteine-NaOH. Two hundred microliters of decontaminated sputa were inoculated on each of two glycerol Lowenstein Jensen (LJ) slopes. Cultures were incubated at 37°C for up to 8 weeks. Positive cultures were confirmed by Ziehl Neelsen smear microscopy and conventional biochemical tests. We used Niacin test, urease, niacin accumulation, p-nitrobenzoic acid (PNB) and p-nitro-alpha-acetylamino-beta-hydroxypropio phenome (NAP) for discrimination of the MTB complex from Mycobacteria other than tuberculosis (MOTT) (Rastogi et al., 1989). Confirmed MTBc were tested for their sensitivity to streptomycin (STR), Isoniazid (INH), Rifampicin (RIF) and Ethambutol (EMB) and isolates were stored in glycerol at -80°C (Canetti et al., 1963).

DNA isolation

Genomic DNA was extracted from TB positive cultures as described elsewhere (van Embden et al., 1993). After spectrophotometry, 10 ng of DNA were used for spoligotyping analysis.

Spoligotyping analysis

Spoligotyping was performed on genomic DNA using commercially available activated Biodine C membranes with the 43 synthetic oligonucleotides covalently bound to the membranes (Ocimum Biosolutions, Huda Techno Enclave Madhapur, India), according to standardized methods as described by Kamerbeek et al. (1997).

Data analysis

The spoligotyping hybridization patterns were converted into binary and octal formats and compared with, previously reported strains in the Spol DB4 database (Brudey et al., 2006). Binary spoligotypes were entered in SITVIT2 database. In 2013, SITVIT2 contained genotyping information of about 75.000 *M. tuberculosis* clinical isolates from different countries. In this database, SIT (Shared International Type) designates spoligotypes shared by two or more *M. tuberculosis* isolates, as opposed to "orphan" which, designates patterns reported for a single isolate.

Detection of Rifampicin and isoniazid resistance

Resistance of isolates to rifampicin and isoniazid was determined by the Genotype MTBDR*plus* Line Probe Assay (LPA) (Hain Lifescience GmbH, Nehren, Germany) according to the manufacturer's instructions (Godreuil et al., 2007).

Single colony plating

Of two suspected mixed strain obtained after spoligotyping were re-cultured into 7H9 broth media, further plated on 7H11 solid medium that incorporates an enzyme that, digests casein to enhance growth of fastidious M.tb, species in single discreet colonies. Singles colonies of interest based on the cell morphology on 7H11 were marked with a felt pen and given a new lab ID.

RESULTS

Study population

The study population of 218 patients included 155 men (71%) and 63 women (29%), out of which five patients

*Corresponding author. E-mail: camaramakhtar@yahoo.fr. Tel: 00221776562436.

(2.2%) tested HIV positive. The age of patients ranged from 18 to 74 years.

Spoligotyping and assignment of mycobacterial lineage and families

Isolates were collected from 218 consecutive smear-positive TB patients out of which 15 (6.8%) did not yield a spoligotype pattern and were confirmed to be MOTT, based on 16S RNA and hsp65 sequencing. Non tuberculosis mycobacteria identified were: *M. fortuitum* 8 (53%), *M. terrae* 1 (6%), *M. intracellulare* 2 (13%), *M. gordonae* 1(6%), *M. abcessus* 1(6%) and two others species which, cannot be fully identified (13%).

Among the remaining 203 *M. tuberculosis* complex isolates, we identified a total of 59 different spoligotypes patterns. The majority of isolates belonged to the Haarlem family (22%), showing the largest spoligotype diversity with 14 different patterns, followed by the T (19%), Beijing (12%), LAM (12%), *M. africanum* West Africa 2 (MAF2) (10%), Cameroon (6%), U (0.4%), EIA5 (1.9%) and X2 (0.9%) (Table 1). Interestingly, 25 isolates (12.3%) were variations of the Manu family.

MTBDR plus results

Of the 203 strains tested by the MTBDR *plus* assay, only two strains (1.0%) showed resistance to isoniazid, and none to rifampicin. Out of the two INH resistant strains, one was a Beijing isolate with an inhA mutation, and the other one was, an EAI isolate with a mutation in katG.

Single colony culture

Of two 'Manu' pattern isolates that were plated to single colonies, for one isolate (D0242) the 'daughter' colonies revealed separate patterns of Lineage 2 (Beijing) and Lineage 4 (Euro-American), while the other 'daughter' isolates remained identical to the 'mother' isolate (Table 2).

DISCUSSION

An interesting finding of the present study is the relatively high proportion (12%) of non-endemic Beijing strains. Such a finding is of general public health interest in Dakar, as Beijing strains are associated with drug resistance, rapid spread and great virulence when compared to other *M. tuberculosis* strains (Gomgnimbou et al., 2012).

Moreover, it was recently suggested that some Manu 2 spoligotypes (which we frequently found in our study), could be the consequence of a "mixed" pattern due to concomitant Beijing and Euro-American strains (the latter comprising H, LAM, X, and T lineages) (Viegas et al., 2010). Therefore, considering the amount of Manu patterns and the possibility of mixed infections in our study, the above mentioned prevalence of Beijing strains could be an underestimation and potentially even be higher than the estimated 12%. Even at 12% prevalence, Dakar already has the highest proportion of TB due to the Beijing family in West Africa. The only other region in West Africa with comparable (though lower numbers) of Beijing strains (10%) is Cotonou, Benin. This phenomenon was identified during the first molecular epidemiological survey in Benin (Affolabi et al., 2009) and later confirmed to be an outbreak of streptomycin resistant Beijing strains in the city (Affolabi et al., 2009). Therefore the high proportion of Beijing strains in 2004 to 2006 is concern in the monitoring of ongoing transmission amongst the current mycobacterial population and their resistance patterns, is important. So far our study suggests that antibiotic resistance was not a major concern in Dakar, as at the time of the study, only two isolates showed INH mono-resistance. Our molecular resistance testing suggests that drug resistance in new TB cases was low in the period of 2004 to 2006, with only 1% isoniazid resistance and no rifampicin resistance. An ongoing nationwide drug resistance survey, will allow us to compare these numbers with current drug resistance levels based on an unbiased sampling of, the entire population.

Our spoligotype results revealed a great diversity of the *M. tuberculosis* strains circulating in Dakar where we not only found the most common global spoligotypes (Brudey et al., 2006) but also the *M. africanum* West Africa 2 lineage, exclusively present in the region. Specifically, these include the Haarlem family followed by T, LAM, Beijing, and MAF2 families. Interestingly, we did not identify any *M. bovis* isolates, which is in line with a previous observation by Diop et al. from 1970 (Diop et al., 1976). The described composition of the MTBc overall confirms the findings from a study, analyzing 69 Senegalese *M. tuberculosis* isolates collected between September 1994 and September 1995, as comparable to our study and the major identified families were the same (Niang et al., 1999).

A major difference between the present and previous studies is in regards to the prevalence of MAF2 in Dakar. A total of 20% of all pulmonary tuberculosis cases in 1970 were still infected by MAF2 (Diop et al., 1976), indicating a slow decrease of the MAF prevalence in Dakar. The present study showed a 10% prevalence of MAF2 in tuberculosis patients between 2004 to 2006, which is similar to 10% in the study conducted in 1995. Countries such as Guinea Bissau, Benin and Nigeria observe high MAF prevalence up to 40% (Kallenius et al., 1999; Affolabi et al., 2009), while a decrease of *M. africanum* prevalence has been observed in other West and Central African countries like Cameroon and Burkina

Table 1. Shows the different Spoligotypes pattern identified by a SIT in the SITVIT database.

Spoligotype description [a]	Octal code	Major Lineage[b]	Family[c]	N° of isolates percentage (%)	Suspect strains	Mixed
□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■■	000000000003771	East Asian (Beijing)	Beijing	25 (12.31)	No	
□□□□□□□□□□□□□□□□□□□□□□□□□□□■□□□□□□■□□□□■■■■■■■	000000004020771	Euro-American	H2	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777777607760771	Euro-American	LAM	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□■□□□□■■■■■■■	777777774020771	Euro-American	H1	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■	777777777720771	Euro-American	H3	22 (10.83)	No	
■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777777777760771	Euro-American	T	24 (11.82)	No	
■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□■■■■■■■■■	777777777763771	Indo-Oceanic	Manu2	4 (1.97)	Yes	
■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■□■■■	777777607760731	Euro-American	LAM4	16 (7.88)	No	
■■■■■■■■■■■■■■■■■■■■■□□□■■■■■■■□□□□■■■■■■■	777777743760771	Euro-American	Cameroon	13 (6.4)	No	
■■■■■■■■■■■■■■■■■■■■■■□□□□□□■□□□□■■■□■■■	777777774020731	Euro-American	H1	2 (0.98)	No	
■■■■■■■■■■■□□□□■■■■■■■■■■■■■■■□□□□■■■■■■■	777703777760771	Euro-American	T	5 (2.46)	No	
■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□■■■■■	777777777700771	Euro-American	T	1 (0.49)	No	
■■■■■■■■■■■■■■■■□■■■■■■■■■■■■■□□□□■■□□□□■	777776777760601	Euro-American	X2	1 (0.49)	No	
■■■■■■□□□■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■■■■	770777777777671	West African 2	AFRI_2	21 (10.34)	No	
■■■■■■■■■■■■■■■■■■□□□□□□■□□□□□□■□□□□■■■■■■■	777777004020771	Euro-American	H1	4 (1.97)	No	
■■■■■■■■■■■■■■■■■□■■■■■■■■■■□■□□□□■■■■■■■	777777377720771	Euro-American	H3	4 (1.97)	No	
■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■□□□□■	777777777760601	Euro-American	T	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■□■■■■	777777777760671	Euro-American	T	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■□■■■■■■■■■■□□□□■■■■■■■	777777677760771	Euro-American	T	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■■□□□□□□■□□□□■■■□■■■	777777770020731	Euro-American	H1	1 (0.49)	No	
■■□■■■■■■■■■■■■■■■■■■■■■■■■□□□□■□■■■■■■■■■	677777777413771	Indo-Oceanic	EAI	1 (0.49)	No	
■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■□□□□■■■■■■■■	777757777760771	Euro-American	T	1 (0.49)	No	
■■■■■■■■■■□□□□■■■■■■■■□■■■■■■■■□□□□□□□□□□□□□	777417737700000	Euro-American	X2	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■□□□□■■■□□■■	777777777720711	Euro-American	H3	1 (0.49)	No	
■■■■■■■■□□□■■■□□□■□□□□□□□□□□□□□□□□□□□□□■■■■	776161000000071	Indo-Oceanic	U	1 (0.49)	No	
■■□■■■■■■■■■□■■■■■■■□□□□■■■■■■■■□□□□■■■■□■■	677737607760751	Euro-American	LAM2	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■■■□■■■■■■□■□□□□■■■■■■■	777777767720771	Euro-American	H3	1 (0.49)	No	
■■■■■■■■■■■■□■■■■■■■■■■■■■■■■□■□□□□■■■■■■■	777757777720771	Euro-American	H3	4 (1.97)	No	
■■■■■■■■■■■□■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	777677607760771	Euro-American	LAM	1 (0.49)	No	
■■□□■■■■■■■■■■■■■□■■■■■■■■■■□■□□□□■■■■■■■	637777377720771	Euro-American	H3	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■□□■■■	777777777760631	Euro-American	T	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□■■■■■■■■■	777777607763771	Indo-Oceanic	Manu2	2 (0.98)	Yes	
■■■■■■■□■■■■■■■■■■■■■■■■■■■■□■□□□□■■■■■■■	776777777720771	Euro-American	H3	1 (0.49)	No	
■■■■■■■■■■■■■■■■■■■■■■■■□□□□□□■□□□□■■■■□■■	777777774020751	Euro-American	H1	1 (0.49)	No	
■■□□□■■■■■■■■■■■■■■□□□□■■■■■■■■□□□□■■■■■■■	617777607760771	Euro-American	LAM1	1 (0.49)	No	

Table 1. Contd.

■■■■■■■■□■■■■■■■■■■■□□□□■■□□□□□■□□□□■■■■■■■	776777606020771	Euro-American	LAM11-ZWE	1 (0.49)	No
■■■■■■■■■■■■■■■■■■■■■□□□■■■■■■■■■□□□□■■■■■■■	777777617760771	Euro-American	T	1 (0.49)	No
■■■■■■■■□■■■■■■■■■■■□□□□□■■□□□□■□□□□■■■■■■■	776777603020771	Euro-American	LAM11-ZWE	1 (0.49)	No
■■■■■■■■□□□□□□■■■■■■■□□□□■■□□□□□■□□□□■■■■■■■	776017606020771	Euro-American	LAM12-Madrid1	1 (0.49)	No
■■■■■■■□■□■■■■■■■■■■□□□□■■□□□□□■□□□□■■■■■■■	775377606020771	Euro-American	LAM11-ZWE	1 (0.49)	No
■■■■■■■■■■■■■■■■■■■■□□□□□■■■■■■■■□□□□■■■■■■■	775377606020771	Indo-Oceanic	Manu3	1 (0.49)	No
■■■■■■■■■■■■■□□■■■■■□□■■■■■■■■□■□□□□■■□■■■■	777747637720671	Euro-American	H3	1 (0.49)	No
■■■■■■■■■□■■■□□■■■■■□□□■■■■■■■■■□□□□□■■□□■■	777347617760311	Euro-American	T	1 (0.49)	No
■■■■■■■■□■■■■■■■■■■■□□□□□■■□□□□■□□□□■■■■■■■	776777603020771	Euro-American	LAM11-ZWE	1 (0.49)	No
■■■■■■■■■■□□□□□□□□□□□□□□■■■■■■■□■□□□□■■■■■■■	777400017720771	Euro-American	H	1 (0.49)	No
■■■■■■■■■■■■■□■■■■■□■□■■■■■■■■■□□■■■■■■■■■	777767657763771	Indo-Oceanic	Manu2	1 (0.49)	yes
■■■■■■■■■■■■■□□■■■■□□□■■■■■■■■■□□□□□■■□■■■	777763617760331	Euro-American	T	1 (0.49)	No
■■■■■■■■■■■■■■■■■■■□□■■■■■■■■■□□■■■■■■■■■	777777637763771	Indo-Oceanic	Manu2	1 (0.49)	yes
■■■■■■■■■■■■■■■■■■■□■□■■■■■■■■■□□■■■■■■■■	777777657771771	Indo-Oceanic	Manu3	1 (0.49)	yes
■■■■■■■■■■■■■■■■■■■■■□□■■■■■■■■□□■■■■■■■■■	777777747763771	Indo-Oceanic	Manu2	1 (0.49)	yes
■□□■■■■■■■■■■■■■□■■■■■■□□□□□□□□□□□□□□□□□□□	477776770000000	Indo-Oceanic	EAI3-IND	1 (0.49)	No
■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■□□□□■■■■■■■	777777703760771	Euro-American	T	1 (0.49)	No
■■■■■■■■■□■■■■□■■■■■■■■■■■■■■■■■□□■■■■□■■■■	777357777763671	Indo-Oceanic	Manu2	2 (0.98)	Yes
■□□■■■■■■■■■■■■■□■■■■■■□□□□□□□□□□□□□□□□□□□	477776770000000	Indo-Oceanic	EAI3-IND	1 (0.49)	No
■■■□□■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■□□□□■	717777777760601	Euro-American	T	1 (0.49)	No
■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□■■■■■■■■■■	777777777767771	Indo-Oceanic	Manu2	12 (5.91)	Yes
■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■■□□■□■■■■■■■	777757777762771	Indo-Oceanic	Manu2	1 (0.49)	Yes
■■■■■■□□■□□□■□□■□■■■□□□■■□□□□□□□□□■■■■■■■■■	771045614003771	Indo-Oceanic	EAI	1 (0.49)	Yes

a: The black and white boxes indicate the presence and absence, respectively, of the specific spacer at positions 1-43 in the DR locus b: Major lineage according to SpolDB4 database c : Family designation according to SpolDB4 database.

Faso (Huet et al., 1971; Ledru et al., 1996; Godreuil et al., 2007; Affolabi et al., 2009; Gehre et al., 2013). However, latest studies described 20% MAF2 prevalence in Burkina and 9% of MAF1 in Cameroon (Niobe-Eyangoh et al., 2003; Gomgnimbou et al., 2012). This reduction could be due to the recent expansion of the so-called *M. tuberculosis* Cameroon family in the country (Niobe-Eyangoh et al., 2003). Interestingly, the Cameroon family was already found in a proportion of 6% in our study indicating that, this family is successfully spreading across West African countries including Senegal**.** The reason for the relative decline of *M. africanum* in West Africa could be due to a lower transmission capacity or lower virulence. Although the virulence capacity of MAF2 strains is not well known, it was already demonstrated that these bacteria are overall attenuated and less immunogenic than *M. tuberculosis* sensu stricto isolates (de Jong et al., 2006; Bold et al., 2012). Another reason for the decline of *M. africanum* in West Africa could be that, certain *M. tuberculosis* lineages possess advantages in their ability to generate secondary cases within a community, and therefore outgrow *M. africanum* in the community (Gehre et al., 2013). The main limitation of our study is that, the geographical sampling area was limited to the Greater Dakar area and therefore is not a representative of the entire country.

Table 2. Spacer analysis of mixed infection test from single colonies.

Original Lab ID	Spoligotype signature [a]	Octal group	Major Lineage[b]
D0242	■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■■□□■■■■■■■■■	777 777 607 763 771	Indo-Oceanic
Single colony ID			
A	Blank		
B	Blank		
C	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■■	000 000 000 003 771	East Asian (Beijing)
D	■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777 777 777 760 771	Euro-American
E	■■■■■■■■■■■■■□■■■■■■■■■■■■■■■■■□□□□■■■■■■■	777 767 777 760 771	Euro-American
F	Blank		
G	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■■	000 000 000 003 771	East Asian (Beijing)
H	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■■■■■■	000 000 000 003 771	East Asian (Beijing)
I	□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□■■■■□■■■■	000 000 000 003 671	East Asian (Beijing)
J	Blank		
DNA Neg.Control2	Blank		

A to **J** distinct colonies that differ morphologically by size and pigmentation. a: The black and white boxes indicate the presence and absence, respectively, of the specific spacer at positions 1-43 in the DR locusb: Major lineage according to SpolDB4 database.

It is recommended that the sampling should be extended to remote areas of Senegal that would allow the establishment of the population structure of *M. tuberculosis* in the whole country and follow the prevalence of Beijing, Lam 10 Cam and MAF2 one decade after isolation of the strains described here. Moreover, the used spoligotyping technique provides a broad overview of genotype variability and circulate *M. tuberculosis* strains in a certain area, yet has a fairly low discriminatory power (Brudey et al., 2006) and needs to be associated with other high resolution techniques such as, the MIRU-VNTR typing or sequenced-based SNP analysis if, transmission dynamics of a potential Beijing outbreak are the focus of a future study.

Conclusion

The genetic variability of the *M. tuberculosis* complex in Dakar reflects a predominance of Euro-American *M. tuberculosis* (Haarlem, T and LAM) lineage with lower prevalence of *M. africanum* West African 2 than previously described. Further investigations focusing on a larger collection of strains covering Senegal nationwide are necessary to describe the population structure of these *M. tuberculosis* strains more accurately and monitor the development of the increased spread of Beijing strains in the country.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The AFTBVAC project study was conducted under funding from the European Commission, Framework Programme 5, Project QOL 2001-01613. We are grateful to WANETAM-project funded by EDTCP which support experiment of this study.

REFERENCES

Affolabi D, Anyo G, Faihun F, Sanoussi N, Shamputa IC, Rigouts L, Kestens L, Anagonou S, Portaels F (2009).First Molecular Epidemiological Study Of Tuberculosis In Benin. Int. J. Tuberc. Lung Dis. 13(3):317-322.

Affolabi D, Faihun F, Sanoussi N, Anyo G, Shamputa IC, Rigouts L, Kestens L, Anagonou S, Portaels F (2009). Possible Outbreak Of Streptomycin-Resistant Mycobacterium Tuberculosis Beijing In Benin. Emerg. Infect. Dis. 15(7):1123-1125.

Bold TD, Davis DC, Penberthy KK, Cox LM, Ernst JD, De Jong BC (2012).Impaired Fitness Of Mycobacterium Africanum Despite Secretion Of ESAT-6. J. Infect. Dis. 205(6):984-990.

Brudey K, Driscoll JR, Rigouts L, Prodinger WM, Gori A, Al-Hajoj SA, Allix C, Aristimuno L, Arora J, Baumanis V, Binder L, Cafrune P, Cataldi A, Cheong S, Diel R, Ellermeier C, Evans JT, Fauville-Dufaux M, Ferdinand S, Garcia De Viedma D, Garzelli C, Gazzola L, Gomes HM, Guttierez MC, Hawkey PM, Van Helden PD, Kadival GV, Kreiswirth BN, Kremer K, Kubin M, Kulkarni SP, Liens B, Lillebaek T, Ho ML, Martin C, Martin C, Mokrousov I, Narvskaia O, Ngeow YF, Naumann L, Niemann S, Parwati I, Rahim Z, Rasolofo-Razanamparany V, Rasolonavalona T, Rossetti ML, Rusch-Gerdes S, Sajduda A, Samper S, Shemyakin IG, Singh UB, Somoskovi A, Skuce RA, Van Soolingen D, Streicher EM, Suffys PN, Tortoli E, Tracevska T, Vincent V, Victor TC, Warren RM, Yap SF, Zaman K, Portaels F, Rastogi N, Sola C (2006).Mycobacterium Tuberculosis Complex Genetic Diversity: Mining The Fourth International Spoligotyping Database (Spoldb4) For Classification, Population Genetics And Epidemiology. BMC Microbiol. 6:23.

Canetti G, Froman S, Grosset J, Hauduroy P, Langerova M, Mahler HT, Meissner G, Mitchison DA, Sula L (1963).Mycobacteria: Laboratory Methods For Testing Drug Sensitivity And Resistance. Bull World Health Organ. 29:565-578.

De Jong BC, Hill PC, Brookes RH, Gagneux S, Jeffries DJ, Otu JK, Donkor SA, Fox A, Mcadam KP, Small PM, Adegbola RA (2006).Mycobacterium Africanum Elicits An Attenuated T Cell Response To Early Secreted Antigenic Target, 6 Kda, In Patients

With Tuberculosis And Their Household Contacts. J. Infect. Dis. 193(9):1279-1286.
Diop S, De Medeiros D, De Medeiros G, Baylet R, Sankale M (1976).Incidence And Geographic Distribution Of Mycobacterium Africanum In Senegal]. Bull. Soc. Med. Afr. Noire Lang. Fr. 21(1):50-56.
Gehre F, Antonio M, Faihun F, Odoun M, Uwizeye C, De Rijk P, De Jong BC, Affolabi D (2013).The First Phylogeographic Population Structure And Analysis Of Transmission Dynamics Of M. Africanum West African 1--Combining Molecular Data From Benin, Nigeria And Sierra Leone. Plos One 8(10):E77000.
Gehre F, Antonio M, Otu JK, Sallah N, Secka O, Faal T, Owiafe P, Sutherland JS, Adetifa IM, Ota MO, Kampmann B, Corrah T, De Jong BC (2013). Immunogenic *Mycobacterium Africanum* Strains Associated With Ongoing Transmission In The Gambia. Emerg. Infect. Dis. 19(10):1598-1604.
Godreuil S, Torrea G, Terru D, Chevenet F, Diagbouga S, Supply P, Van De Perre P, Carriere C, Banuls AL (2007).First Molecular Epidemiology Study Of Mycobacterium Tuberculosis In Burkina Faso. J. Clin. Microbiol. 45(3):921-927.
Gomgnimbou MK, Refregier G, Diagbouga SP, Adama S, Kabore A, Ouiminga A, Sola C (2012).Spoligotyping Of Mycobacterium Africanum, Burkina Faso. Emerg. Infect. Dis. 18(1):117-119.
Huet M, Rist N, Boube G, Potier D (1971).[Bacteriological Study Of Tuberculosis In Cameroon]. Rev Tuberc Pneumol (Paris). 35(4):413-426.
Kallenius G, Koivula T, Ghebremichael S, Hoffner SE, Norberg R, Svensson E, Dias F, Marklund BI, Svenson SB (1999).Evolution And Clonal Traits Of Mycobacterium Tuberculosis Complex In Guinea-Bissau. J. Clin. Microbiol. 37(12):3872-3878.
Kamerbeek J, Schouls L, Kolk A, Van Agterveld M, Van Soolingen D, Kuijper S, Bunschoten A, Molhuizen H, Shaw R, Goyal M, Van Embden J (1997).Simultaneous Detection And Strain Differentiation Of Mycobacterium Tuberculosis For Diagnosis And Epidemiology. J. Clin. Microbiol. 35(4):907-914.
Ledru S, Cauchoix B, Yameogo M, Zoubga A, Lamande-Chiron J, Portaels F, Chiron JP (1996).Impact Of Short-Course Therapy On Tuberculosis Drug Resistance In South-West Burkina Faso. Tuber Lung Dis. 77(5):429-436.
Niang MN, De La Salmoniere YG, Samb A, Hane AA, Cisse MF, Gicquel B, Perraut R (1999).Characterization Of M. Tuberculosis Strains From West African Patients By Spoligotyping. Microbes Infect. 1(14):1189-1192.
Niobe-Eyangoh SN, Kuaban C, Sorlin P, Cunin P, Thonnon J, Sola C, Rastogi N, Vincent V, Gutierrez MC (2003). Genetic Biodiversity Of Mycobacterium Tuberculosis Complex Strains From Patients With Pulmonary Tuberculosis In Cameroon. J. Clin. Microbiol. 41(6):2547-2553.
Rastogi N, Goh KS, David HL (1989).Selective Inhibition Of The Mycobacterium Tuberculosis Complex By P-Nitro-Alpha-Acetylamino-Beta-Hydroxypropio Phenone (NAP) And P-Nitrobenzoic Acid (PNB) Used In 7H11 Agar Medium. Res. Microbiol. 140(6):419-423.
Van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, Mcadam R, Shinnick TM, Et Al. (1993).Strain Identification Of Mycobacterium Tuberculosis By DNA Fingerprinting: Recommendations For A Standardized Methodology. J. Clin. Microbiol. 31(2):406-409.
Viegas SO, Machado A, Groenheit R, Ghebremichael S, Pennhag A, Gudo PS, Cuna Z, Miotto P, Hill V, Marrufo T, Cirillo DM, Rastogi N, Kallenius G, Koivula T (2010).Molecular Diversity Of Mycobacterium Tuberculosis Isolates From Patients With Pulmonary Tuberculosis In Mozambique. BMC Microbiol. 10:195.
WHO (2015). Global Tuberculosis Report 2015. [Cited 2015 02012016]; Available at: Http://Www.Who.Int/Tb/Publications/Global_Report/Fr/