

First molecular epidemiological study of tuberculosis in Benin

D. Affolabi,^{*,†} G. Anyo,[†] F. Faihun,^{*} N. Sanoussi,^{*} I. C. Shamputa,[†] L. Rigouts,[†] L. Kestens,[‡]
S. Anagonou,^{*} F. Portaels[†]

^{*}Laboratoire de Référence des Mycobactéries, Cotonou, Bénin; [†]Mycobacteriology Unit and [‡]Immunology Unit, Institute of Tropical Medicine, Antwerp, Belgium

SUMMARY

OBJECTIVES: To assess the diversity of *Mycobacterium tuberculosis* strains in Cotonou, Benin, and the risk factors associated with clustering.

METHODS: We analysed one sputum sample from 194 consecutive new pulmonary tuberculosis (TB) cases using two genotyping methods: spoligotyping and the 12 loci mycobacterial interspersed repetitive unit–variable number of tandem repeats (MIRU-VNTR). The data obtained were compared to the SpolDB4.0 database.

RESULTS: We have found that spoligotype 61, highly predominant in West Africa, was also the most prevalent

strain in Cotonou. We observed that the Beijing family represented 10.3% of strains and was associated with resistance to streptomycin. We also confirmed that combining spoligotyping and MIRU-VNTR provided a higher discriminatory power than the two techniques used individually.

CONCLUSION: Spoligotype 61 and Beijing genotype are the most prevalent genotypes of *M. tuberculosis* in Cotonou.

KEY WORDS: molecular epidemiology; *Mycobacterium tuberculosis*; Cotonou

OF THE ESTIMATED 8.8 million new tuberculosis (TB) cases and 1.6 million annual deaths attributed to TB worldwide,¹ most occur in low-resource countries, particularly in Africa, where the TB situation is worsened by social conditions, the human immunodeficiency virus (HIV) epidemic and the emergence of multidrug-resistant (MDR) strains (defined as resistance to at least isoniazid [INH] and rifampicin [RMP]).

Benin is a country in West Africa with an estimated population of 8.5 million. Cotonou is the largest city in Benin and accounts for about one third of the TB cases detected in the country. The DOTS strategy has been implemented in Benin for several years, and covers the whole country. According to the World Health Organization (WHO), the estimated TB incidence rate increased from 80 per 100 000 population in 1990 to 88/100 000 in 2005, while the rate of detected cases remained nearly the same (40/100 000 in 1990 compared to 39/100 000 in 2005).¹ In 2006, HIV seroprevalence in the general population was 1.8%,² whereas the rate among TB patients was 11% (National TB Programme [NTP] report, unpublished data).

As in most resource-poor countries, TB epidemiology in Benin has so far largely consisted of reporting the number of cases detected and their demographic data. Little is known about the molecular epidemiology of TB in this country; for example, the SpolDB4.0

spoligotyping database published only four strains of the 39 609 entries from Benin.³ Molecular epidemiology could, however, help us to better understand the TB transmission dynamics for the control of the disease in Benin.

Numerous methods for *Mycobacterium tuberculosis* genotyping have been developed; the reference method is restriction fragment length polymorphism of the insertion sequence 6110 (IS6110-RFLP).^{4,5} However, this method is labour-intensive, has poor discriminatory power for strains with low copy numbers of IS6110 and requires large quantities of DNA. Spoligotyping is a more simple polymerase chain reaction (PCR) based method, but is less discriminatory than IS6110-RFLP.⁶ Another PCR-based method, the mycobacterial interspersed repetitive unit–variable number of tandem repeats (MIRU-VNTR),⁷ based on 12 independent loci, has been widely used and has a discriminatory power comparable to IS6110-RFLP when combined with spoligotyping.^{8–10} Although the resolution of MIRU-VNTR has recently increased by the use of 15 loci,¹¹ this new combination of loci has to be further evaluated.

This paper presents the results of the first molecular epidemiological study conducted out on TB isolates from patients in Benin to assess the diversity of *M. tuberculosis* strains in Cotonou using two genotypic methods, spoligotyping and the 12 loci MIRU-VNTR.

Correspondence to: Dissou Affolabi, Laboratoire de Référence des Mycobactéries 01, BP 817 Cotonou, Bénin. Tel: (+229) 21 33 15 33. Fax: (+229) 21 33 70 57. e-mail: affolabi_dissou@yahoo.fr

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MATERIALS AND METHODS

Patients

Between July 2005 and October 2006, specimens from 201 new smear-positive TB cases were included in the study. All patients resided in Cotonou or its suburbs. Two sputum samples were collected and analysed per patient, but only one positive culture per patient was used for drug susceptibility testing (DST) and DNA fingerprinting. Demographic data and a blood sample for HIV testing were collected from each patient. HIV testing was performed using an enzyme-linked immunosorbent assay (ELISA). Seropositive samples were confirmed by a discriminatory HIV1/2 test (Genie II HIV1/HIV2®, BioRad, France).

Culture and DST

Two sputum samples from each patient were decontaminated using the modified Petroff method¹² and cultured in the manual Mycobacteria Growth Indicator Tube (MGIT) (Becton Dickinson, Sparks, MD, USA),¹³ and on Löwenstein-Jensen (LJ) media. The *M. tuberculosis* isolates (one per patient) were tested for susceptibility against RMP, INH, streptomycin (SM) and ethambutol (EMB) using the proportion method on LJ medium at the following respective concentrations: 40 µg/ml, 0.2 µg/ml, 4 µg/ml and 2 µg/ml.¹⁴ Quality assurance of DST was performed annually by the Institute of Tropical Medicine in Antwerp, Belgium.

DNA fingerprinting

DNA was extracted by transferring two drops of MGIT-positive cultures into 300 µl of 10 mM Tris-HCl and 1 mM EDTA (1 × TE) and killing the bacteria by boiling the suspension for 5 min. Spoligotyping was performed as previously described,⁶ and the data obtained were compared to the SpolDB4.0 database.³ MIRU-VNTR was performed by individually amplifying each of the 12 loci as previously described, and results were combined into digit allelic profiles.⁷

M. tuberculosis H37Rv was used as a positive control, and water and reagent control were used as negative controls.

Statistical analysis

All data were analysed using SPSS version 9.05 (SPSS Inc, Chicago, IL, USA) and Epi Info 6.02 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Crude relative risks (RR) and their 95% confidence intervals (95% CI) were calculated to evaluate the association between clustering or Beijing strains and different factors. Contingency tables were analysed using the Pearson's χ^2 test and differences were considered significant if $P < 0.05$.

A backward elimination procedure based on the likelihood ratio was used to select variables to be included in logistic regression models. Adjusted odds ratios (ORs) and their 95% CIs were derived from the

final logistic models. For these models goodness-of-fit was assessed using the Hosmer and Lemeshow test.

A cluster was defined as two or more strains with identical spoligotype and MIRU-VNTR patterns.

Ethical consideration

All patients gave informed consent. The study was approved by the Institutional Review Board of the NTP of Benin.

RESULTS

Study population

Of the 201 newly recruited pulmonary TB cases, five samples that were culture-negative or contaminated and two showing a combination of double alleles in several MIRU-VNTR loci, suggestive of mixed infection, were excluded from the analysis. The female-to-male ratio was 3.4; the age of the patients ranged from 17 to 69 years, with a median age of 30 years. HIV serology testing results were available for 190 patients, of whom 27 (14.2%) were HIV-1 seropositive, comprising 10 females (37%) and 17 males (63%). The remaining 163 patients (85.8%) were HIV-negative.

Drug susceptibility testing

DST results were available for 143 strains, of which two (1.4%) were MDR, six (4.2%) were resistant to INH, two (1.4%) were resistant to EMB and 29 (20.3%) were resistant to SM. In total, 34 strains (23.8%) were resistant to at least one drug and 109 strains (76.2%) were pan-susceptible (Table 1). Of the two patients infected by MDR strains, one was HIV-positive, lived abroad and had come back to Benin a few months before being diagnosed with TB. The second MDR patient lived permanently in Benin and was HIV-negative.

Spoligotyping

A total of 95 distinct spoligotypes were identified; 126 strains (64.9%) were grouped into 27 clusters,

Table 1 Resistance patterns of strains

Type of resistance	n (%)
Monoresistant	
INH	3 (2.1)
SM	28 (19.6)
Polyresistant	
INH + RMP	1 (0.7)
INH + EMB	1 (0.7)
INH + RMP + SM + EMB	1 (0.7)
Other patterns	0
Total INH + RMP (MDR)	2 (1.4)
Total resistant	34 (23.8)
Susceptible to all drugs	109 (76.2)
Total	143

INH = isoniazid; SM = streptomycin; RMP = rifampicin; EMB = ethambutol; MDR = multidrug-resistant.

Table 2 Spoligotypes

Spoligotypes	Patterns	n (%)
Already described spoligotypes	33	113 (58.2)
Clusters	16	96 (49.5)
Unique	17	17 (8.8)
New described spoligotypes	62	81 (41.8)
Clusters	11	30 (15.5)
Unique	51	51 (26.3)
Total unique	68	68 (35.1)
Total clusters	27	126 (64.9)
Total	95	194

with cluster sizes ranging from 2 to 40. Comparing the spoligotypes obtained with those in the international database SpolDB4, the three most frequent clusters were ST 61, ST 1 and ST 438, containing respectively 40, 17 and 8 strains (Table 2).

Of the 194 strains studied, 62 new spoligotypes not yet described in the database were identified; they were distributed into 51 unique patterns and 11 clusters of 30 strains (one cluster of seven strains, one of four strains, one of three strains and eight of two strains) (Table 2).

MIRU-VNTR

Among the 194 strains, 74 distinct MIRU-VNTR patterns were detected and 150 (77.3%) strains were grouped into 30 clusters, with cluster sizes ranging from 2 to 20 strains.

Spoligotyping plus MIRU-VNTR

By combining spoligotyping and MIRU-VNTR, 66 strains (34.0%) were grouped into 14 clusters. Of the 40 strains with ST 61, the combination of spoligotyping and MIRU-VNTR identified 12 unique patterns and 28 strains grouped into four clusters with 15, 7, 4 and 2 strains. All strains with ST 1 (Beijing family) shared the same type by MIRU-VNTR. Neither of the two MDR strains was grouped into a cluster (Figure).

We also compared the characteristics age, sex, HIV status and drug resistance between clustered and non-clustered strains. In univariate analysis, no association was found between age, sex or HIV status and clustering; however, drug-resistant strains were more likely to be clustered than drug-susceptible strains (Table 3). Indeed, 42.6% of clustered strains showed resistance to at least one drug tested while only 12.4% of non-clustered strains were resistant ($P < 0.001$, RR = 2.38, 95%CI 1.63–3.47) (Table 3). In multivariate analysis, only resistance to at least one drug appeared to be significantly associated with clustering (adjusted OR = 4.71, 95%CI 2.06–10.77). Furthermore, Beijing strains were more likely to be associated with resistance to at least one drug tested ($P < 0.001$, RR = 7.34, 95%CI 4.59–11.74).

Mono-resistance to SM, the most common pattern of resistance, was found in all of the 17 ST 1 strains

Table 3 Crude RR and 95%CI of clustering in patients

Epidemiological characteristic	Patients infected by:		Crude RR (95%CI)	P value
	Clustered strains n (%)	Non-clustered strains n (%)		
Male sex	51 (77.3)	99 (77.3)	1.00 (0.62–1.59)	NS
HIV-positive	12 (19.0)	15 (11.8)	1.42 (0.88–2.29)	NS
Age <30 years	33 (50.0)	58 (45.3)	1.13 (0.77–1.67)	NS
Drug-resistant	23 (42.6)	11 (12.4)	2.38 (1.63–3.47)	<0.001

RR = relative risk; CI = confidence interval; NS = not significant; HIV = human immunodeficiency virus.

with DST, one of the two strains with ST 255, five of the 38 ST 61 strains with DST, the ST1 540 strain and seven of the 70 non-clustered strains.

DISCUSSION

This study was conducted to obtain a first insight into the molecular epidemiology of TB in Cotonou, Benin, and the risk factors associated with clustering in this city.

As in other studies,^{9,15} these results show that combining spoligotyping and MIRU-VNTR provides a higher discriminatory power than the two techniques taken individually. We have therefore chosen the combination of these two techniques to analyse the biodiversity of *M. tuberculosis* strains in Cotonou.

Using this combination, we found a relatively low level of clustering of strains (34%), suggesting a low rate of recent transmission of TB. A study performed in Tanzania using IS6110-RFLP showed a similar percentage (33%), whereas other African studies have shown a higher percentage of clustering, such as the recent study in the neighbouring country of Burkina Faso, using the same molecular methods, with 53.3% clustered strains.^{9,15–22} Although the proportion of clustered strains in a population is assumed to reflect the rate of recent transmission of TB, this hypothesis is sometimes questionable. In our setting, the low level of clustered strains, comparable to that in industrialised countries,^{23–25} could partly be attributed not only to the preponderance of reactivation of old infections but also to the efforts made by the NTP for early detection of TB cases.

The comparison of spoligotypes found in this study with the International Spoligotyping Database showed that the most prevalent spoligotype was ST 61, which belongs to the Latino-American and Mediterranean family (LAM) family.³ This genotype was previously described to be prevalent in countries on the West African coast, and according to these results it was suggested that strains with this genotype have recently expanded in West Africa.^{9,26–28}

No association was found between clustering and epidemiological data such as age, sex and HIV status. Numerous studies, especially in industrialised

countries, have identified factors such as young age, male sex and HIV status to be associated with clustering, in contrast to developing countries,²⁹⁻³¹ where several studies found no association between these fac-

tors and clustering.^{9,15} Although the only two MDR strains reported herein were not in a cluster, there was a significant association between drug resistance and clustering, especially within the W-Beijing family.

Spoligotype patterns	Patients n	MIRU patterns	Spoligotype	Spoligotype patterns	Patients n	MIRU patterns	Spoligotype
7 0 0 0 7 6 7 7 7 7 6 0 7 0 0	1	224424242231	200	7 7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223325153322	61
7 0 0 0 0 0 7 6 5 6 7 4 1 7 1	1	224434244221	*	7 7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223315163321	61
7 7 4 0 7 7 7 7 7 7 7 0 7 1	3	224424244221	438	7 7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223424244221	61
7 7 4 0 7 7 7 7 7 7 7 0 7 1	1	224524244221	438	7 7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	224424244221	61
7 7 4 0 7 7 7 7 7 7 7 0 7 1	1	224424254221	438	7 7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	224224243221	61
7 7 4 0 7 7 7 7 7 7 7 0 7 1	1	224524234221	438	7 7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	227234263521	61
7 7 4 0 7 7 7 7 7 7 7 0 7 1	1	221524244221	438	7 7 7 7 7 7 7 4 3 7 6 0 7 3 1	1	223315153323	403
7 7 4 0 7 7 7 7 7 7 7 0 7 1	1	223225133324	438	7 1 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223315153321	*
7 7 4 0 7 7 7 7 7 7 5 7 7 0 7 1	1	224424254221	*1	7 7 7 7 7 7 7 4 3 7 6 0 7 5 1	1	223315133321	838
7 7 4 0 7 7 7 7 7 7 5 7 7 0 7 1	1	227524244221	*1	7 7 7 7 7 7 7 6 7 7 6 4 7 7 1	1	223315153321	*
7 7 4 0 6 7 7 7 7 7 7 0 7 1	1	224524244221	*	7 7 7 7 7 7 0 3 4 3 7 4 0 7 7 1	1	223315153223	*8
7 3 4 0 7 7 7 7 7 7 7 1 0 7 1	2	224424244221	*2	7 7 7 7 7 7 0 3 4 3 7 4 0 7 7 1	1	223315153323	*8
7 7 4 0 7 7 7 7 7 7 7 5 0 7 1	1	224424242221	437	7 7 7 7 7 7 0 3 4 3 7 6 0 7 7 1	1	223315153223	*
7 7 4 0 7 7 7 7 7 7 7 5 0 7 1	1	224325153323	437	7 7 7 7 7 7 7 4 3 7 4 0 1 7 1	1	223215143313	*
0 0 0 0 0 7 7 7 7 7 7 5 0 7 1	1	224424242221	*	7 6 1 3 7 7 7 4 3 7 6 0 7 3 1	1	223315153323	*9
7 1 4 0 7 7 7 7 7 7 7 0 0 1 1	1	224524244221	*	7 6 1 3 7 7 7 4 3 7 6 0 7 3 1	1	223323143322	*9
7 7 4 0 7 7 6 0 7 7 7 7 0 3 1	1	225424244221	330	7 7 7 7 7 7 7 7 7 7 6 0 7 7 1	1	223325143336	53
7 7 4 0 7 7 6 0 7 7 7 7 0 3 1	1	224414244221	330	7 7 7 7 7 7 7 7 7 7 6 0 7 7 1	1	225325153324	53
7 7 4 0 7 7 6 0 7 7 7 7 0 7 1	1	224424243221	331	7 7 7 7 7 7 7 7 7 7 6 0 7 7 1	1	225125113322	53
7 7 4 0 7 7 6 0 7 7 7 7 0 7 1	1	224424244221	331	6 5 7 7 7 7 7 7 7 7 2 0 7 7 1	1	223325143336	*
7 7 4 0 7 7 6 0 7 7 7 7 0 7 1	1	226224233522	331	7 7 7 7 7 7 4 7 7 7 6 0 7 7 1	1	223325143321	451
7 7 4 0 7 7 6 0 7 7 6 1 0 3 1	1	224424244221	*	7 7 7 3 7 7 7 7 7 7 6 0 7 7 1	1	223425143428	1166
5 3 4 0 7 7 6 0 7 7 7 7 0 7 1	1	224424243231	*	6 7 7 7 3 7 7 3 7 7 6 0 7 7 1	1	223225133323	*
7 7 0 0 0 3 7 0 7 7 7 7 0 0 1	1	224424243221	*	7 3 5 7 7 7 7 7 7 7 6 0 7 3 1	2	223325153221	432
7 7 0 0 0 3 6 0 7 7 7 7 0 7 1	1	224424243221	*	7 3 7 7 7 7 7 7 7 7 6 0 7 3 1	1	223325153221	838
7 7 4 0 4 5 6 6 7 7 7 7 5 6 0 1	1	223414244221	*	7 7 7 7 7 7 7 7 7 7 5 6 0 7 7 1	1	223328151321	462
7 7 4 2 7 7 7 7 7 7 7 0 7 1	1	223215143323	*	7 7 7 7 7 7 7 7 7 7 2 0 7 7 1	4	225325153323	50
7 7 4 1 7 7 7 7 7 7 7 6 7 1	1	223315153321	*	7 7 7 7 7 7 7 7 7 7 2 0 5 7 1	1	225325153323	463
7 7 0 3 7 7 7 7 7 7 7 6 7 1	1	223315153321	*3	7 7 7 7 7 6 7 7 7 7 6 0 7 7 1	1	223325153322	119
7 7 0 3 7 7 7 7 7 7 7 6 7 1	1	222315133321	*3	7 7 7 7 7 7 7 7 7 7 2 0 7 3 1	1	225313154323	49
7 7 0 7 7 7 7 7 7 7 7 6 7 1	1	225424243522	326	7 7 7 7 7 7 7 7 7 7 2 0 7 3 1	1	225313153323	49
7 7 0 7 7 7 7 7 7 7 7 6 7 1	1	224424243422	326	7 7 7 7 7 7 7 7 7 7 2 0 6 7 1	2	224323153223	168
7 7 0 3 7 7 7 0 7 7 7 7 6 3 1	1	225424243522	*	7 7 7 3 7 7 7 7 7 7 2 0 6 7 1	1	224323153223	*
7 7 7 3 7 7 7 4 3 7 6 7 7 7 1	1	225424243522	*	7 7 7 7 3 7 7 7 7 7 2 0 7 3 1	1	223325153323	1135
7 7 0 7 7 4 0 3 7 7 7 7 6 7 1	1	227224253521	*	7 7 7 7 2 1 7 7 7 7 2 0 7 3 1	1	223325153323	*
7 7 0 7 7 7 7 7 7 7 7 6 7 1	1	227224242521	*	7 5 7 3 7 7 7 4 3 7 6 0 7 7 1	1	223215143323	*
7 7 4 3 3 7 7 7 7 7 3 7 6 7 1	1	236224233522	*	7 7 7 3 7 7 7 0 3 7 6 0 7 7 1	1	224524243221	*
7 7 0 2 5 5 6 6 7 7 3 7 2 6 0 1	1	236224233522	*	7 6 1 7 7 7 7 4 3 7 6 0 7 3 1	1	224424244221	844
0 7 4 0 6 7 6 0 6 7 7 7 0 7 1	1	214123243221	*	7 3 7 3 6 7 7 7 7 7 6 0 6 6 1	1	224425144446	*
7 7 4 0 0 0 0 7 7 7 7 7 0 7 1	1	124424244221	*	0 7 7 7 7 7 7 6 0 2 0 7 3 1	1	223425143323	*
7 7 4 0 0 0 0 7 7 7 7 7 0 3 1	1	124424244221	146	7 7 7 7 7 7 7 7 7 7 7 7 7 1	1	222424244221	523
7 7 4 0 0 0 0 7 7 7 7 7 0 3 1	1	224524243221	146	7 7 7 7 7 7 7 7 7 7 4 1 3 7 3 1	1	225424244221	48
5 7 4 0 0 0 0 7 7 7 7 7 0 3 1	1	124424244221	*	6 7 7 7 7 7 7 7 7 4 1 3 7 7 1	1	255325223433	342
7 7 4 0 0 0 2 7 7 7 7 2 3 1	1	124424244221	*	6 7 7 7 7 7 7 3 7 4 1 3 7 7 1	1	255324223433	342
7 7 4 0 0 0 0 7 7 7 7 2 3 1	1	124424244221	*	6 7 7 7 7 7 7 3 7 4 1 3 7 7 1	1	254325223433	342
7 7 4 0 0 0 7 7 7 7 7 0 3 1	1	124424244221	*	6 7 7 7 7 7 7 7 7 4 5 3 7 7 1	1	255325223433	*
7 7 4 0 4 0 0 7 7 7 7 7 0 7 1	2	222414244221	*4	6 7 7 7 7 7 7 3 7 4 1 3 7 7 1	1	255325223433	*
7 7 4 0 4 0 0 6 7 7 7 7 0 7 1	1	221424244221	*	7 7 7 7 4 0 0 1 7 7 6 0 7 7 1	1	125326153222	159
7 1 0 0 4 4 0 1 7 3 7 7 0 6 1	1	225424244221	*	6 7 7 7 7 4 7 6 0 0 0 1 3 1	1	223425151321	*
7 1 0 0 4 7 7 7 7 7 7 6 6 1	1	226224233522	*	7 7 7 7 6 7 7 0 0 0 0 0 0 1	1	325225153323	450
7 7 0 0 4 7 7 7 7 7 7 6 6 1	1	224424243221	*	7 7 7 7 7 0 0 0 0 0 0 0 7 3 1	1	212325153324	1204
7 0 0 0 1 7 7 7 7 7 6 4 0 2	2	225424243522	*5	7 7 7 7 5 0 0 0 0 0 0 0 7 3 1	1	233325153223	*
7 7 4 0 7 4 0 0 4 0 3 7 0 7 1	1	224424244221	*6	7 7 4 0 7 7 4 0 0 6 0 3 0 3 1	1	224524243221	*
7 7 4 0 7 4 0 0 4 0 3 7 0 7 1	1	225424244221	*6	7 7 4 1 7 7 4 0 1 7 2 3 0 3 1	1	221424244221	*
7 7 4 0 7 4 0 0 4 0 3 7 0 7 1	1	225524244221	*6	7 7 4 0 7 7 6 0 0 0 0 0 0 3 1	3	224424244221	*10
7 7 4 0 7 4 0 0 4 0 3 7 0 7 1	1	224414234221	*6	3 7 4 0 7 7 6 0 0 0 0 0 0 1 1	1	224414244221	*
7 7 4 0 7 4 0 0 4 0 3 7 4 7 1	1	225424244221	*	7 7 4 1 7 7 7 0 0 0 0 0 0 3 1	1	224424244221	*
7 3 4 0 7 4 0 0 4 1 3 7 0 7 1	1	225424244221	*	7 7 0 0 0 3 4 0 0 0 0 0 3 0 7 1	2	224524243221	*11
7 7 4 0 7 4 0 0 4 0 3 7 0 6 0 1	1	225424244221	*7	7 7 0 0 0 3 4 0 0 0 0 0 3 0 7 1	1	224424243221	*11
7 7 4 0 7 4 0 0 4 0 3 7 0 6 0 1	1	223325163533	*7	7 7 0 0 0 3 4 0 0 0 0 0 3 0 7 1	1	224524233221	*11
7 7 4 0 7 7 4 0 4 3 7 7 0 3 1	1	222315153323	*	7 7 0 0 0 3 4 0 0 0 0 0 3 0 7 1	1	224424244221	*11
7 7 7 7 7 7 4 3 7 6 0 7 7 1	7	223315153321	61	7 7 0 0 0 3 4 0 0 0 0 0 3 0 7 1	1	124424244221	*11
7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223215153321	61	7 7 0 0 0 3 4 0 0 0 0 0 3 0 7 1	1	223315153323	*11
7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223315153021	61	4 0 0 0 0 3 7 4 3 7 6 0 7 7 1	1	223315153323	852
7 7 7 7 7 7 4 3 7 6 0 7 7 1	15	223315153323	61	0 0 0 0 0 0 0 0 0 7 2 0 7 3 1	1	225313153323	830
7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223315152324	61	7 7 4 0 4 0 0 7 0 0 0 0 0 0 1	1	224424254221	*
7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223315153324	61	0 0 0 0 0 0 0 0 0 0 0 3 7 7 1	17	223325163533	1
7 7 7 7 7 7 4 3 7 6 0 7 7 1	4	223215153323	61	0 0 0 0 0 0 0 0 0 0 0 3 6 7 1	1	223325163533	255
7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223215143323	61	0 0 0 0 0 0 0 0 0 0 0 3 6 7 1	1	225424244221	255
7 7 7 7 7 7 4 3 7 6 0 7 7 1	1	223215153313	61	0 0 0 0 0 0 0 0 0 0 0 3 0 3 1	1	223325163533	540
7 7 7 7 7 7 4 3 7 6 0 7 7 1	2	223315133323	61				

Figure Combination of spoligotypes and MIRU types. * Spoligotypes not yet described. MIRU = mycobacterial interspersed repetitive unit.

W-Beijing family strains have been found to be very rare in countries on the West African coast.^{9,26,28} In this study, the proportion was 10.3% and almost all the strains were resistant to SM. According to the patient information, most of those whose isolates harboured Beijing strains lived in the same area in Cotonou. As this area is not properly mapped out, it was difficult to confirm an epidemiological link between these patients. However, investigations are ongoing to further characterise the strains and to map patients' residence using the Geographical Information System (GIS). W-Beijing family strains have been associated with drug resistance;³² moreover, its rapid spread in some settings suggests that this family is intrinsically virulent.

In the present study, as we recently showed, the prevalence of MDR among new cases has been found to be low in Cotonou, particularly in patients who habitually lived in Cotonou.³³ This is probably due to the strict application of the DOTS strategy in Benin over several years. The levels of drug resistance found in the present study are similar to those found in Cotonou in 2004: overall resistance 25%, MDR 1.6% and resistance to SM 20.1%.³³ The relatively high level of resistance to SM found in Cotonou, and also in several other African countries, is probably related to the wide use of low-cost aminoglycosides to treat respiratory infections other than TB.²⁸

Only smear-positive specimens were included in this study; it is, however, possible that the results could be slightly different if smear-negative, culture-positive specimens were also included.

In conclusion, ST 61, which is highly predominant in *M. tuberculosis* strains in West Africa, is the most prevalent genotype in Cotonou, followed by the Beijing family, which is associated with drug resistance in this population and occurs in up to 10.3% of all strains identified.

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R É S U M É

OBJECTIF : Evaluer la diversité génétique des souches de *Mycobacterium tuberculosis* à Cotonou, Bénin, et les facteurs de risque associés au regroupement des souches en grappes.

MÉTHODES : Nous avons analysé les échantillons d'expectoration provenant de 194 nouveaux cas de tuberculose pulmonaire à microscopie positive admis de façon consécutive. Un échantillon par patient a été analysé au moyen de deux tests génotypiques : le spoligotypage et le « mycobacterial interspersed repetitive unit-variable number of tandem repeats » (MIRU-VNTR) utilisant 12 loci. Les profils des souches obtenus ont ensuite été

comparés à ceux de la base de données internationale SpolDB4.0.

RÉSULTATS : Nous avons observé que le spoligotype le plus fréquent à Cotonou est le spoligotype 61 suivi du type « Beijing », qui représente 10,3% des souches et qui est, par ailleurs, associé à la résistance à la streptomycine. Nous avons par ailleurs confirmé que la combinaison du spoligotypage et du MIRU-VNTR est plus discriminante que chacun des tests pris isolément.

CONCLUSION : Le spoligotype 61 et le type « Beijing » sont les génotypes les plus fréquents à Cotonou.

R E S U M E N

OBJETIVOS : Evaluar la diversidad de las cepas de *Mycobacterium tuberculosis* en Cotonou, Benín, y los factores de riesgo que se asocian con su distribución en conglomerados.

MÉTODOS : Se analizó una muestra de esputo de cada uno de los 194 casos nuevos de tuberculosis pulmonar que se presentaron en forma consecutiva, mediante dos métodos de genotipado : con oligonucleótidos que reconocen un locus con repeticiones directas conservadas (spoligotyping) y un análisis de las unidades intergénicas repetitivas micobacterianas (número variable de repeticiones en tándem) (MIRU-VNTR) en 12 locus polimórficos. Los datos obtenidos se compararon con la base de datos SpolDB4.0.

RESULTADOS : Se observó por espiligotipado que el genotipo 61, muy predominante en África occidental, era también el más frecuente en las cepas de Cotonou. La familia Beijing representó el 10,3% de las cepas y se asoció con resistencia a estreptomycina. Asimismo, se confirmó que combinando el espiligotipado con el análisis MIRU-VNR se alcanza un mayor poder discriminatorio que con cada una de las técnicas utilizada separadamente.

CONCLUSIÓN : El espiligotipo 61 y el genotipo Beijing constituyen los genotipos más frecuentes de *M. tuberculosis* en Cotonou.