## Spoligotyping of Mycobacterium africanum, Burkina Faso

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Using Ziehl-Neelsen–positive slides collected from tuberculosis diagnostic centers in Burkina Faso, we showed that 20% of 80 spoligotyping-positive DNA samples had a characteristic *Mycobacterium africanum*–specific genomic signature. This result suggests that *M. africanum* is still present in Burkina Faso at almost the same prevalence as 15–20 years ago.

Mycobacterium africanum remains a major pathogen in Africa (1). Recently, de Jong et al. estimated the prevalence of M. africanum to be 1.7% in Burkina Faso (1); their estimate was based on a 2007 study by Godreuil et al., who unexpectedly did not identify any M. africanum isolate within a collection of 120 M. tuberculosis complex clinical isolates in 2001 from 79 tuberculosis patients living in Ouagadougou and 41 living in Bobo Dioulasso, the 2 largest cities in the country (2). However, 2 patterns (isolates 94 and 90) can be recognized as M. africanum by their characteristic spoligotyping signature (deletion of spacers, 8, 9, and 39) and an association of mycobacterial interspersed repetitive unit (MIRU)  $24 \ge 2$ , MIRU31  $\ge 4$ , and MIRU40 < 3 signature (2,3).

In the neighboring country of Ghana (which has 200 km of common borders with Burkina Faso), another study suggested that the population structure of *M. tuberculosis* complex comprises 1) 34% spoligo-international type (SIT) 61 (named the Cameroon clade, also present in Burkina Faso); 2) 30% *M. africanum* (including *M. africanum* West African 1 and West African 2); and 3) 36% principal genetic group 2 and 3 modern strains (e.g., T, U [unknown], Haarlem, X, LAM [Latino-American and Mediterranean]), with minor prevalence of other principal genetic groups, i.e., the East-African Indian, Beijing, and *M. bovis* clades (4,5). These observations—and their congruence to

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estimates by Ledru et al. in 1996 of an 18.4% prevalence of *M. africanum* strains isolated from the 300 patients in whom tuberculosis was newly diagnosed in Burkina Faso during 1992–1994 (5)—prompted us to reexamine the conclusions of Godreuil et al. on the *M. africanum* prevalence in Burkina Faso.

#### The Study

The study, which we conducted during March–September 2010, had 3 goals. First, we wanted to determine whether we could extract DNA and perform high-throughput spoligotyping on a Luminex 200 device (Luminex, Austin, TX, USA) on acid-fast bacillus–positive slides (6). Second, we wanted to reestimate the prevalence of *M. africanum* in Burkina Faso from a recent and random sample of slides. Third, we wanted to further analyze the relative proportion of *M. africanum* West African I and West African 2 strains in Burkina Faso because this country is part of central western Africa, where the 2 *M. africanum* West African 1 and 2 strains are present at various relative rates (2). We report on all the goals of this project, even though goal 3 remains to be confirmed because of the small sample size.

From within 14 geographically independent centers in Burkina Faso (Figure), we recruited a random sample of 186 Ziehl-Neelsen (ZN) slides that had been included in a national study on drug resistance, as approved by the ethical committee for health research in Burkina Faso (2007–031; June 28, 2009). Of 186 DNA samples extracted from as many ZN slides, 143 sputum samples had been scored 3+, 18 were scored 2+, 10 were scored 1+, 5 had 1–9 bacilli total (±), totaling 176 positive slides from as many sputum samples. In addition, test results were negative for 9 and unknown for 1.

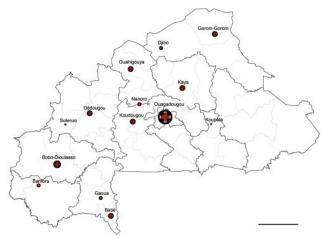


Figure. Origin of samples described in study of *Mycobacterium africanum* in Burkina Faso. Dark outlined borders indicate province; light outlined borders indicate regions. Scale bar = 100 km. Sources: Institut Géographique du Burkina Faso/Centre Muraz-PNT.

In a preliminary trial of 9 independent 3+ positive slides, DNA extraction was attempted by 2 methods: an enzymatic method (7) and a classical thermic lysis in a Chelex suspension (InstaGene; Bio-Rad, Hercules, CA, USA) (8). In our study, only the Chelex method produced good results, i.e., enabled us to obtain DNA that was successfully PCR amplified and produced a full spoligotyping pattern (results not shown). The quantity of DNA extracted was superior for all tests by the enzymatic lysis (n = 3) as by the Chelex (n = 6), as estimated by spectrophotometry (NanoDrop ND-1000; LabTech, Ringmer, UK). Thus, DNA can be successfully extracted by the enzymatic method for many human or bacterial cells but not for M. tuberculosis complex because no spoligotype could be obtained. We therefore analyzed the 176 experimental slides by using the Chelex extraction procedure.

The origins of all ZN slides assessed in this study are shown in the Figure, and genotyping results are shown in the Table (full results in the online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-0275-Techapp.pdf). As observed in a much larger set of samples from Ghana, the Cameroon family (SIT61) also prevails in Burkina Faso (18 [25%] isolates) (4). Whether the Cameroon strains from Burkina Faso are similar or identical to those from Ghana remains to be studied.

Our result confirms the observation by Godreuil et al. in 2007 on the prevalence of the SIT61/Cameroon strains in Burkina Faso (2). However, we detected 16 *M. africanum* spoligotypes (West African 1 and West African

Table. Distribution of classified genotypes of *Mycobacterium tuberculosis* complex, Burkina Faso\*

No. (%) isolates,
n = 72‡
18 (25)
14 (19)
4 (6)
16 (22.2)
10 (13.9)
2 (2.8)
1 (1.4)
1 (1.4)
2 (2.8)
10 (13.9)
7 (9.7)
3 (4.2)
4 (5.6)
1 (1.4)
16 (22.2)
1 (1.4)
5 (6.9)
1 (1.4)
1 (1.4)

<sup>\*</sup>CAM, Cameroon; SIT, spoligo-international type; WA, West African; CAS, Central Asian; LAM, Latino American–Mediterranean.

2), i.e., a minimal M. africanum prevalence of 20%, close to 18.4% found by Ledru et al. in 1996 (5). Third, the T and Haarlem strains represented 16 (22%) and 10 (14%), respectively, of the patterns; other genotypes were rare (5 CAS [Central Asian], 4 X, 1 M. bovis, 1 Beijing, 1 LAM). Finally, the relative prevalence of M. africanum West African 1 from M. africanum West African 2 could first be assessed by the spoligotyping signature (2 vs. 14; online Technical Appendix). Specific single nucleotide polymorphism detection could constitute another classification tool for M. africanum sublineages (10,11). Unfortunately, detection of katG203 single nucleotide polymorphism failed on the slide-extracted DNAs (results not shown), and our study is limited by a suboptimal yield in positive spoligotyping results (80 [43%] of 186), an issue that should be improved.

#### **Conclusions**

The results of our study diverge on the M. africanum prevalence in Burkina Faso from results from Godreuil et al. (2) (1.9% vs. 20%). These authors were intrigued to not identify more M. africanum isolates and suggested that their finding might reflect "a decrease in M. africanum prevalence in these countries," referring to a similar decrease in Cameroon during 1971-2003 (12,13). We believe that in the study by Godreuil et al., an unintentional bias was introduced against M. africanum, given the difficulty of isolating this genotypic variant in routine practice in mycobacteriologic laboratories. Differences in M. africanum prevalence in culture-based and sputum-based studies might reflect the difficulties of growing and isolating M. africanum in some national TB reference laboratories in western Africa. M. africanum, which is closely related to M. bovis, has peculiar growing requirements that are not always satisfied. Supplementation of Löwenstein-Jensen medium with pyruvate is mandatory and not standardized (from 0.1% to 0.4%).

The pyruvate requirements of some members of the *M. tuberculosis* complex were recently shown to be caused by a mutation creating an inactive pyruvate kinase (14). This specific mutation of *M. africanum* has major implications for its metabolism and growth.

Implementation of adequate culture and molecular identification facilities in Burkina Faso are needed. A potential solution to avoiding the bias from culture and from DNA extraction from slides could be to extract DNA directly from sputum, e.g., by storing surplus sputum prospectively in 70% ethanol. Additional work also is needed to improve analytical methods for ZN slides to refine description of *M. tuberculosis* genetic diversity and eventually to provide predictive genetic drug susceptibility testing. Introduction of newer and faster TB diagnostic methods are urgently needed in this area of western Africa.

<sup>†</sup>Described in (9).

<sup>‡</sup>Excludes 4 new and 4 unclassified genotypes.

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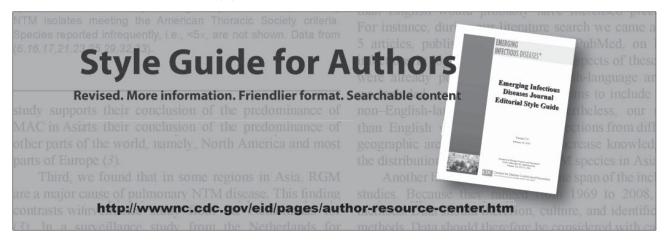
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# Spoligotyping of *Mycobacterium africanum*, Burkina Faso

### **Technical Appendix**

181	SIT*	No.	Spoligo binary	Clade	Geographic origin	Center
ND   028B (3+)   028B (3+)   028B (3+)   028B (3+)   028B (3+)   038B (3+)	ND	137C (3+)		West African 1	unknown	
181   0.49A (3+)	ND	108B (3+)		West African 1	Ouagadougou	CNLAT
181	ND	028B (3+)		West African 2	Gorom Gorom	Gorom Gorom
187   080A (3+)	181	049A (3+)		West African 2	Gorom Gorom	Gorom Gorom
ND	181	059A (3+)		West African 2	Dédougou	Dédougou
326   133A (3+)	187	080A (3+)		West African 2	Bobo Dioulasso	CRLAT
326   177A (3+)	ND	095B (3+)		West African 2	Kaya	Kaya
ND   234B (3+)	326	133A (3+)		West African 2	Ouagadougou	CNLAT
Second (3+)	326	177A (3+)		West African 2		
326 318A (3+) ND 531A (3+) 187 548A (3+) 326 594C (3+) 10 027A (3+) 11 027A (3+) 12 17 A (3+) 13 18 18 18 18 18 18 18 18 18 18 18 18 18	ND	234B (3+)		West African 2	Bobo Dioulasso	CRLAT
181   403A (3+)	326	280A (3+)		West African 2	Banfora	Banfora
ND   531A (3+)	326	318A (3+)		West African 2	Dédougou	Dédougou
187         548A (3+)         Bobo Dioulasso         CRLAT           326         594C (3+)         Bobo Dioulasso         Dédougou         CAM         Batié         Banfora         CAM         Diametra         Banfora         Banfora         Banfora         Batié		403A (3+)		West African 2	Nanoro	
326   594C (3+)	ND	531A (3+)		West African 2	Ouagadougou	Paul VI
1 027A (3+)	187	548A (3+)		West African 2	Bobo Dioulasso	CRLAT
991 271A (3+)	326	594C (3+)		West African 2	Dédougou	Dédougou
ND         010B (3+)         Bobo -Dioulasso         CMA Dafra           61         020A (3+)         Banfora         Banfora           61         026B (3+)         Batié         Batié           61         084A (3+)         CAM         Ouagadougou         CMA Pissy           ND         087A (3+)         CAM         Ouahigouya         Ouahigouya           61         094A (3+)         CAM         Ouahigouya         Ouahigouya           61         094A (3+)         CAM         Nanoro         Nanoro           61         112A (3+)         CAM         Batié         Batié           61         180B (3+)         CAM         Ouagadougou         CNLAT           61         278A (3+)         CAM         CAM         CAM         Kaya         Kaya           61         278A (3+)         CAM         CAM         CAM         Koudougou         Koudougou           61         426A (3+)         CAM         CAM         Gaoua         Gaoua           61         420A (3+)         CAM         CAM         Ouagadougou         CNLAT           61         420A (3+)         CAM         Ouagadougou         CMA         CAM           61 <td>1</td> <td>027A (3+)</td> <td></td> <td>Beijing</td> <td>Batié</td> <td>Batié</td>	1	027A (3+)		Beijing	Batié	Batié
61 020A (3+)	991	271A (3+)		M. bovis 1		CMA Pissy
61 026B (3+) 61 084A (3+) ND 087A (3+) 61 094A (3+) 61 112A (3+) 61 180B (3+) 61 204B (3+) 61 2078A (3+) 61 278A (3+) 61 426A (3+) 61 426A (3+) 61 420A (3+) 61 580A (3+) 61 580A (3+) 61 094A (3+) 61 094A (3+) 62 034B (3+) 63 034B (3+) 64 034B (3+) 65 034B (3+) 66 034B (3+) 67 034B (3+) 68 034B (3+) 69 034B (3+) 60 034B (3+) 60 034B (3+) 61 034B (3+) 62 034B (3+) 63 034B (3+) 64 034B (3+) 65 034B (3+) 66 034B (3+) 67 034B (3+) 68 034B (3+) 69 034B (3+) 69 034B (3+) 60 034B (3+) 61 0	ND	010B (3+)			Bobo -Dioulasso	CMA Dafra
61         084A (3+)         084A (3+)         000000000000000000000000000000000000	61	020A (3+)		CAM	Banfora	Banfora
ND         087A (3+)         087A (3+)         087A (3+)         087A (3+)         094A	61	026B (3+)		CAM	Batié	Batié
61 094A (3+)	61	084A (3+)		CAM	Ouagadougou	CMA Pissy
61 112A (3+)	ND	087A (3+)		CAM	Ouahigouya	Ouahigouya
61 180B (3+)	61	094A (3+)		CAM	Nanoro	Nanoro
61 204B (3+)	61	112A (3+)		CAM	Batié	
61 278A (3+)	61	180B (3+)		CAM	Ouagadougou	CNLAT
61 426A (3+)	61	204B (3+)		CAM	Kaya	Kaya
61 474A (3+)	61	278A (3+)			Koudougou	Koudougou
61 420A (3+)	61	426A (3+)		-	Banfora	Banfora
61 580A (3+)	61	474A (3+)				
	61			-	Ouagadougou	CNLAT
	61	580A (3+)		CAM	Ouagadougou	CMA Pissy
	57	592C (3+)		CAM	Ouagadougou	CNLAT
2550 602A (3+)    Solution    CAM Bobo Dioulasso CRLAT	2550	602A (3+)		CAM	Bobo Dioulasso	CRLAT
61 605A (3+) Bobo Dioulasso CRLAT	61				Bobo Dioulasso	CRLAT
61 611A (3+)	61	611A (3+)		CAM	Ouahigouya	Ouahigouya
1327 079B (3+)    Solution Solution    CAS1_Delhi Bobo Dioulasso CRLAT	1327	079B (3+)		CAS1_Delhi	Bobo Dioulasso	CRLAT
ND 085A (3+)	ND	085A (3+)				Djibo
ND 129A (3+)					Djibo	
1327 188A (3+)    Second 1327 188A (3+)    Second 1327 188A (3+)    CAS1_Delhi Ouagadougou CMA 30	-	188A (3+)				
1327 481A (3+)    ■■■□□□□■■■■□□■■■■■■■■■■□□□□□□□□□□□□	1327	481A (3+)		CAS1_Delhi	Ouagadougou	CNLAT
47 038B (3+)	47	038B (3+)		H1	Djibo	Djibo

SIT*	No.	Spoligo binary	Clade	Geographic origin	Center
47	054A (3+)		H1	Nanoro	Nanoro
47	099B (3+)		H1	Ouagadougou	CMA30
47	132B (3+)		H1	Ouagadougou	CNLAT
47	386A (3+)		H1	Ouagadougou	CNLAT
47	397A (3+)		H1	Koupéla	Koupéla
47	583B (3+)		H1	Koudougou	Koudougou
50	535A (2+)		H3	Solenzo	Solenzo
49	606C (3+)		H3	Dédougou	Dédougou
50	609A (3+)		H3	Ouahigouya	Ouahigouya
42	522A (3+)		LAM9	Gaoua	Gaoua
ND	206A (3+)		New	Batié	Batié
ND	232B (3+)		New	Solenzo	Solenzo
ND	455A (3+)		New	Koudougou	Koudougou
ND	589B (3+)		New	Gorom Gorom	Gorom Gorom
53	051A (3+)		T1	Ouagadougou	CMA 30
462	187A (3+)		T1	Ouagadougou	CMA 30
53	438A (IC)		T1	Kaya	Kaya
53	480C (3+)		T1	Ouagadougou	CNLAT
53	495A (2+)		T1	Ouagadougou	PPH Yalgado
804	533B (3+)		T1	Koudougou	Koudougou
53	534B (3+)		T1	Ouagadougou	PPH Yalgado
53	547A (3+)		T1	Bobo Dioulasso	CRLAT
53	549A (3+)		T1	Bobo Dioulasso	CRLAT
53	607A (3+)		T1	Kaya	Kaya
78	062A (3+)		T1-T2	Bobo Dioulasso	CMA Dafra
78	601A (3+)		T1-T2	Bobo Dioulasso	CRLAT
52	585B (3+)		T2	Ouagadougou	CMA 30
504	582B (3+)		T3	Koudougou	Koudougou
1227	078A (3+)		T5_MAD2	Bobo Dioulasso	CMA Dafra
1227	490A (3+)		T5_MAD2	Ouagadougou	CMA Pissy
ND	145A (3+)		U	Gorom Gorom	Gorom Gorom
1200	190B (3+)		U	Gorom Gorom	Gorom Gorom
2398	213A (3+)		U	Bobo Dioulasso	CRLAT
1200	508A (3+)		U	Ouagadougou	CNLAT
2397	159B (3+)		X3	Gorom Gorom	Gorom Gorom
2397	430A (3+)		X3	Ouagadougou	CNLAT
2397	510B (3+)		X3	Ouagadougou	CNLAT
200	608A (3+)		Х3	Ouahigouya	Ouahigouya

<sup>\*</sup>SIT, spoligotyping international type (14); CRLAT, Centre Régional de Lutte Antituberculeuse; CNLAT, Centre National de Lutte Antituberculeuse; PPH, Service de Pneumo-Phtisiologie; CMA, Centre Médical Antenne Chirurgicale.

Absence