Abstract

Background: To identify strains of Mycobacterium tuberculosis complex (MTBc) circulating in Bamako region during the past 10 years. Methods: From 2006 to 2016, we conducted a cross-sectional study to identify with spoligotyping, clinical isolates from tuberculosis (TB)-infected patients at different stages of their treatments in Bamako, Mali. Results: Among the 904 suspected TB patients included in the study and thereafter tested in our BSL-3 laboratory, 492 (54.4%) had MTBc and therefore underwent spoligotyping. Overall, three subspecies, i.e., MTB T1 (31.9%) and MTB LAM10 (15.3%) from lineage 4 and T1 strain was the most prevalent. Furthermore, the data indicate an increasing proportion of primary drug resistance overtime in Bamako. Conclusion: This study showed a high genetic diversity of strains isolated in Bamako region and highlights that Mycobacterium tuberculosis T1 strain was the most prevalent. Furthermore, the data indicate an increasing proportion of primary drug resistance overtime in Bamako.

Keywords: 10 years, bamako, spoligotyping, strain distribution, tuberculosis

INTRODUCTION

Tuberculosis (TB) has been a global threat to human health and it remains a major public health burden in developing countries. The World Health Organization (WHO) reports in 2015 that one-third of world’s population is infected by M. tuberculosis, 10.4 million people fell ill of TB, and 1.4 million people died despite its curability when early detected and properly treated.[1] TB has recently been dramatically expanded due to the human immunodeficiency virus (HIV)/AIDS and the emergence of multidrug-resistant (MDR) strains.[2] Although TB is a worldwide concern, >85% of cases are seen in Asia and Africa, respectively, with 55% and 30%, and >95% of global TB-related deaths occurred in those low- and middle-income countries.[1] In Mali, a landlocked country, the prevalence and incidence rates of TB were, respectively, 91 and 58 for 100,000 habitants according to the WHO in 2014; 5809 new TB patients were notified among of which 132 MDR or chronic-TB patients were reported.[1] While the prevalence and incidence rates are decreasing since 2000, there is an increase of drug resistance (DR) within the country mainly the primary MDR-TB which moved from 2.5% in 2011 to 3.4% in 2016.[3,4] Thus, the increased rate of drug-resistant and MDR strains remains a serious concern for TB control in Mali.

Recently, mycobacterial strain typing has greatly facilitated and improved the epidemiology of TB.[5] Strain typing is an interesting tool in understanding TB distribution and has a potential in formulating appropriate strategies for the disease control in a particular region. In a previous study in Bamako,
Mali, Traore et al. reported a predominancy of MTB T1, MTB LAM, and Mycobacterium africanum (MAF) 2 from 126 isolates collected between 2006 and 2010. Thus, the aim of this study was to characterize the global strain diversity and the prevalence of DR profiles in these strains during the past 10 years.

**METHODS**

**Study design and setting**

Mali is a landlocked West African country with a size of 1,241,248 km² and population of approximately 14.5 million people in 2009. Bamako, the capital city, has a population of approximately two million people and is divided into six urban districts, with each district having a health referral center, where TB diagnostic and treatment services are available. The University Teaching Hospital (UTH) at Point-G is located in district three and serves as the last level of reference for TB. In 2014, more than one-third of the total TB patients in Mali were managed in Bamako. In addition, 80% of the Malian MDR-TB cases were included in this study.

Since 2006, SEREFO BSL-3 laboratory, yearly certified, within the University Clinical Research Center (UCRC) of the University of Sciences, Techniques and Technologies of Bamako (USTTBB) is performing TB culture, drug susceptibility testing (DST), and strain typing. The suspected pulmonary TB patients were enrolled from local reference centers and the UTH at Point-G in Bamako, Mali. Mycobacterial isolates from patients had been freeze. In the present cross-sectional analysis, we focused on baseline strains and their drug susceptibility profiles.

**Study population**

Suspected TB patients were screened at the study sites based on smear-positive result of either Ziehl–Neelsen (ZN) or auramine/rhodamine (A/R) staining, and consented participants were then enrolled into the studies. Only TB patients with Mycobacterium tuberculosis complex (MTBc) infection confirmed by culture and strain-typed (to determine subspecies) during the 10-year study period were included in the final analysis.

Patients were classified based on their TB treatment history as new patients, who had received TB treatment for the first time for <4 weeks and previously treated patients, who had received TB treatment for >4 weeks according to the WHO guidelines. Patients received treatment through their physicians in accordance with national guidelines of the national TB program in Mali. Newly, TB diagnosed patients are treated with a fixed dose combination of 2 months of rifampicin (R), isoniazid (H), pyrazinamide (Z), and ethambutol (E) and 4 months of (R) and (H) (2RHZE/4RH; Cat. 1) while retreatment patients received 2 months of (R), (H), (Z), (E), and streptomycin (S), followed by 1 month with the same combination but without (S) and 5 months of (R), (H), and (E) (2RHZE/1RHZE/5RHE; Cat. 2). Patients clinically suspected of having MDR disease (those who failed Cat. 2 regimen) or confirmed MDR-TB patients underwent a 6-month course of kanamycin (K), ofloxacin (O), ethionamide (Ei), and (Z) during the 6-month inpatient period and followed by 15 months of the same combination without kanamycin (6KOEiZ/15OEiZ). Although approved by the WHO, Mali has not yet started implementation of the short-course 9-month MDR regimen.

**Laboratory tests**

Preenrollment sputum smear microscopy by ZN or fluorescent smear microscopy using A/R (fluorescent microscopy [FM]) at local reference centers was followed by indirect FM and culture at the UCRC-SEREFO BSL-3 laboratory. This TB laboratory is certified by the College of American Pathologists for external quality control. Confirmed MTBc isolates underwent DST and spoligotyping.

**Culture and drug susceptibility testing**

Sputum specimens were digested and decontaminated using the standard N-Acetyl-L-Cysteine/4% NaOH solution, concentrated by centrifugation (4500 rpm), and inoculated on both liquid (Mycobacterium Growth Incubator Tube [BBL™ MGIT™ Becton Dickinson, Sparks MD, USA]), and solid (Middlebrook 7H11 Agar and Selective 7H11 Agar) media. Simultaneously, an aliquot of concentrated specimen was prepared for indirect commercial A/R staining (BBL™ GenProbe, San Diego, CA, USA). Indirect first-line DST was performed on MTBc isolates using MGIT AST/SIRE System (Becton Dickinson, Sparks, MD, USA), and the results were interpreted based on the United States of America (USA) Center for Disease Control (CDC) recommendations for critical drug concentrations. Moreover, second-line DST was performed on randomly selected MDR isolates at collaborating centers.

**Spoligotyping**

Spoligotyping was performed using a commercially available kit (Isogen Life Science, Netherlands). Strain comparisons were made with SPOTCLUST (SpolDB3-based) database following the development of the film. Corresponding shared spoligotypes were further defined using the SITVIT (Institut Pasteur de la Guadeloupe) database.

**Data and statistical analysis**

A descriptive analysis was conducted to determine the median of age with the minimum and maximum age. The proportion of each strain type and their drug susceptibility profiles was calculated. Analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA) and Epi Info September 2002’s version 7 (CDC, Atlanta, USA).

**RESULTS**

**Sociodemographic characteristics**

Of the 445 participants, the median age was 31 years (range 3–78), 104 (23.4%) were women, and 48 (10.8%) were
HIV positive. Three hundred and eight patients (69.2%) were new TB patients while 137 (30.8%) were previously treated. Among the 48 patients coinfected with HIV, 15 (31.1%) had MTB T1 strain, 11 (22.9%) had MAF 2, and 5 (10.4%) had MTB LAM10 infection.

Lineage and family assignments
During the study period, >2100 cultures from 904 suspected TB patients were performed in the UCRC/BSL-3 laboratory, and 492 patient's strains (54.4%) grew as MTBc of whom 445 (90.4%) were typed by spoligotyping. From spoligotyping, three mycobacterial subspecies were identified with 362 (81.3%) of *M. tuberculosis*, 79 (17.8%) of MAF, and 4 (0.9%) of *Mycobacterium bovis*. Furthermore, 25 known strain families were represented in our clinical isolates' collection. The predominant subspecies were MTB T1 with 142 (31.9%) isolates, MAF 2 with 75 (16.8%) isolates, and MTB LAM 10 with 68 (15.3%) isolates [Table 1 and Figure 1]. The other subspecies were all below 5% of prevalence except *M. tuberculosis* family 33, which represented 6.7% [Table 1 and Figure 1].

Prevalence of spoligotype during the past 10 years of follow-up
Although three subspecies dominated during the 10 years of follow-up, we also noticed the appearance of other spoligotypes during the past 5 years (2011–2016) such as MTB H37Rv, MTB Haarlem, MTB family 33, and MTB X families [Table 1].

Drug susceptibility and strain type
Among the 349 (78.4%) isolates tested for TB, first- and second-line DST, 209 (59.9%) were pan sensitive to all drugs tested, and 140 (40.1%) were resistant to at least one drug [Table 3]. The prevalence of MDR-TB was 3.3% and 67.3%, respectively, in patients naive to treatment and previously treated patients [Table 3]. Of the three major families, the proportion of MDR-TB was higher in MTB T1 family with 54 (47.4%) [Table 4], and moreover, the two extensively drug resistance-TB isolates belonged to the MTB T clade [Table 4]. Despite its low number, MTB H37Rv showed a high proportion of MDR strain on 8 (72.7%) [Table 4].

### Table 1: 10-year distribution of spoligotypes from tuberculosis patients in Bamako, Mali

| Spoligotypes | Year | Total (%) |
|--------------|------|-----------|
|              | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 |
| MTB CAS      | 1    | 1    | 1    | 1    | 1    | 2    | 2    | 8 (1.8) | | | |
| *Mycobacterium bovis*-BCG | 1 | 2 | 1 | | | | | 4 (0.9) | | | |
| MTB Beijing  | 2    | 4    | 1    | 1    | | | | | | | |
| MTB EA12     | 1    | 1    | | | | | | | | | |
| MTB EA13     | 1    | | | | | | | | | |
| MTB EA15     | 1    | 1    | 1    | 3    | 2    | 2    | 1    | 11 (2.5) | | | |
| MTB family 33| 1    | 1    | 4    | 8    | | 4    | 9    | 5    | 31 (6.7) | | | |
| MTB H37Rv    | 1    | 1    | 1    | 1    | 6    | 1    | | | 11 (2.5) | | | |
| MTB Haarlem 1| 2    | 2    | 2    | 3    | 1    | | | | 10 (2.4) | | | |
| MTB Haarlem 3| 3    | 1    | | 1    | 2    | 5    | 2    | 3    | 17 (3.8) | | | |
| MTB Haarlem 2| | | | | | | | | 1 (0.2) | | | |
| MTB LAM1     | 1    | | 1    | 1    | 1    | | | 4 (0.9) | | | |
| MTB LAM10    | 2    | 6    | 3    | 2    | 2    | 13   | 18   | 2    | 16   | 4    | 68 (15.3) |
| MTB LAM7     | 1    | | 1    | | | | | | | | 2 (0.4) |
| MTB LAM8     | 1    | | 1    | | 1    | | | | | 3 (0.7) | | |
| MTB LAM9     | 1    | 1    | 1    | | 1    | 4    | 2    | 10   | | 20 (4.5) | | |
| MAF 2        | 7    | 16   | 10   | 2    | 2    | 15   | 9    | 14   | 4    | 75 (16.8) | | |
| MAF 1        | 1    | 1    | | | | | | | | 2 (0.4) | | |
| MTB S        | 1    | | 2    | | | | | | | | 4 (0.9) |
| MTB T1       | 5    | 19   | 10   | 8    | 9    | 12   | 11   | 19   | 10   | 32   | 7   | 142 (31.9) |
| MTB T2       | 1    | 1    | | 1    | | 1    | 1    | | | | | 5 (1.1) |
| MTB T3       | 1    | | | | | | | | | | | 2 (0.4) |
| MTB T4       | 1    | | | | | | | | | | | 3 (0.7) |
| MTB X1       | 1    | | | | | | | | | | | 4 (1.1) |
| MTB X3       | 1    | | | | | | | | | | | 4 (0.9) |
| **Total**    | 18   | 53   | 33   | 15   | 10   | 23   | 48   | 75   | 44   | 103  | 23  | 445 (100) |

Global distribution per year of the 25 spoligotypes identified in Bamako region. Empty cells mean zero (not found). MTB: *Mycobacterium tuberculosis*, CAS: Central Asian, EAI: East African Indian 2; 3; and 5, LAM: Latino American and Mediterranean 1; 7; 8; 9; and 10, MAF: *Mycobacterium africanum* 1 and 2; MTB clade S, T 1, 2, 3 and 4; and clade X 1 and 3, BCG: Bacillus Calmette-Guérin
**Figure 1**: Representation of some Spoligotype patterns for Malian tuberculosis patients. Patterns illustrating data comparison for some tuberculosis patients derived by use of SPOTCLUST (SpolDB3-based) database according to the international octal coding system. January 2006 - December 2016, Bamako, Mali.
| Mtb. T3 | 743760771 | 4 |
| Mtb. T4 | 7777000760751 | 4 |
| Mtb. H37Rv | 77777037700771 | 4 |
| Mtb. Haarlem1 | 77777774020771 | 4 |
| Mtb. Haarlem2 | 4020771 | 4 |
| Mtb. Haarlem3 | 7776777720771 | 4 |
| Mtb. LAM1 | 677767607760770 | 4 |
| Mtb. LAM7 | 7761600000007 | 4 |
| Mtb. LAM8 | 177772000000731 | 4 |
| Mtb. LAM9 | 77777607760771 | 4 |
| Mtb. LAM10 | 7777747743760771 | 4 |
| Mtb. X1 | 7777743760771 | 4 |
| Mtb. X2 | 757747643760771 | 4 |
| Mtb. X3 | 577757743760771 | 4 |
| Mtb. S | 777777473760771 | 4 |
| M. africanum | 774777777777071 | 5 |
| M. africanum | 770777777777761 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 7707777777777671 | 6 |
| M. africanum | 70006663141671 | 6 |
| M. africanum | 510344004442461 | 6 |

Contd...
Table 2: Distribution of major Mycobacterium tuberculosis complex lineages (L1-L6) identified in tuberculosis patients in Bamako region during the past 10 years (2006-2016)

| L               | Family | Number of isolates, n (%) |
|-----------------|--------|---------------------------|
| Indo-Oceanic (L1) | Family 33 | 31 (6.7)                  |
| East Asian (L2)  | Beijing | 8 (1.8)                    |
| CAS (L3)         | CAS     | 8 (1.8)                    |
| Euro-American (L4)| T-clade  | 152 (34.5)                |
|                 | LAM     | 97 (22)                    |
|                 | S-clade | 4 (0.9)                    |
|                 | X-clade | 9 (2.1)                    |
|                 | Haarlem | 28 (6.3)                   |
|                 | H37Rv   | 11 (2.5)                   |
| West African 1 (L5) | MAF WA 1 | 4 (0.9)                    |
| West African 2 (L6) | MAF WA 2 | 75 (17)                    |

MTB complex lineages and families from 441 isolates of patients with tuberculosis in Bamako region, Mali. L: Lineage, EAI: East African Indian, CAS: Central Asian, LAM: Latin American and Mediterranean, MAF WA 1/2: Mycobacterium africanum West African Type 1 and 2, MTB: Mycobacterium tuberculosis

Table 3: Global first- and second-line drug susceptibility profile of isolates recovered from tuberculosis patients in Bamako region between 2006 and 2016

| Drug susceptibility profile | Patients’ category | Total, n (%) |
|-----------------------------|--------------------|--------------|
|                             | New patients, n (%)| Previously treated, n (%)| |
| Pan sensitive               | 195 (79.6)         | 14 (13.5)     | 209 (59.9) |
| Other resistances           | 42 (17.1)          | 18 (17.3)     | 60 (17.2)  |
| MDR-TB                      | 8 (3.3)            | 70 (67.3)     | 78 (22.3)  |
| XDR-TB                      | -                  | 2 (1.9)       | 2 (0.6)    |
| Total                       | 245 (100)          | 104 (100)     | 349 (100)  |

Although Mycobacterium bovis isolates are known intrinsically resistant to pyrazinamide, we did not test in this study. MDR-TB: Multidrug-resistant tuberculosis, XDR-TB: Extensively drug-resistant tuberculosis

Discussion

Lineage 4 and lineage 6 were the dominant causes of TB in Bamako region during the past 10 years, with, respectively, 68.2% and 16.8% of the cases. Recent genetic characterization of M. tuberculosis strains has greatly facilitated and improved understanding of the molecular epidemiology of TB.[10] Spoligotyping appears to have the specific characteristics needed to address both a rapid mycobacterial identification and strain tracking of infections (molecular epidemiology) including investigation of chains of transmission. In fact, this technique is very helpful and allows the concomitant identification and differentiation of M. tuberculosis strains and save time needed to culture as slow-growing bacterium such as M. tuberculosis.[5,10] The clinical utility of spoligotyping is also potentialized by its rapidity in bacterial identification and comparison of strain molecular profiles, which is very useful in surveillance of TB transmission and prevention of further spread of the disease. Although some studies pointed out that spoligotyping technique has low discriminatory value to differentiate between two strains (for chain of transmission investigations), however identifications of MTBc subspecies by spoligotyping (which this study is about) and its comparability to other available techniques like mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR), is greatly recognized.[11,12]

We followed using spoligotyping the distribution of mycobacterial strains isolated from TB patients in Bamako urban region during the past 10 years. Although we noticed that few patients have traveled to Bamako city to receive better care, we have to mention that all were recruited in this region. We observed a high proportion of the three subspecies (MTB T1, MAF 2 and MTB LAM), similarly to what Traore et al. reported in 2012,[1,13] suggesting a relative stability and endemicity of the disease transmission. However, unlike Mali, the continuous predominance of MTB T1 was not observed in our neighboring countries such as Burkina Faso, Benin, and Nigeria. Gehre et al.
It has to be noted that 140 isolates (40.1%) were resistant to at least one TB first-line drug, and the global prevalence of MDR-TB was 3.3% and 67.3%, respectively, in patients naive to treatment and previously treated patients. This seems to be high and is in line with the global increase of primary DR in Mali. In a separate study, 2.9% of newly infected TB patients had resistance to rifampicin after screened by Xpert MTB/RIF. This rate should be taken seriously by the Mali National TB Program to plan further actions to avoid uncontrolled spread of drug-resistant strains in the country.

The number of patients included in this study may not be extrapolated to the whole country as 10,000 new TB cases are detected yearly in Mali with a third in Bamako region where this study was conducted. Despite this limitation, this study is unique in Mali and/or African region in that it collected and identified continuously mycobacterial strains over 10-year period. These data could be used to predict the disease transmission dynamics in the region and to propose a more adapted and effective public health response in the future.

### Conclusion

Genotyping of *M. tuberculosis* strains in Mali showed a high genetic diversity during the past 10 years. *M. tuberculosis* T1 strain within modern lineage Euro-American was the most prevalent. The continuous leading proportion of these strains within *M. tuberculosis* complex during the past 10 years may suggest a stable and conserved host-pathogen interaction. A targeted intervention is thus necessary to overcome this endemic disease in the region.

### Acknowledgment

The authors would like to thank the Division of clinical research of the national institute of health and allergic diseases (Bethesda, MD, USA). We are also grateful to Dr. Traore Breihima, Dr Bindogo PP Dembele, Dr. Ousmane M’Baye, Late Sady Tounkara, and Ms. Mariam H Diallo, for their help and assistance for the study recruitment and laboratory tests.

### Financial support and sponsorship

This study was partially funded by the USTTB through NIH/R01 grant R01AI110386, the Northwestern University (Chicago, IL, USA) through ACTG U01AI069471. B.D. was supported by a TDR fellowship, TIMS ID B40072, the Special Programme for Research and Training in Tropical Diseases, co-sponsored by the UNICEF, UNDP, World Bank, and WHO.

### Conflicts of interest

There are no conflicts of interest.

### References

1. World Health Organization. Global Tuberculosis Report 2015; 2016.
2. World Health Organization. Global Tuberculosis Reports 2013. Geneva, Switzerland: World Health Organization; 2013.
3. Traore B, Diarra B, Dembele BP, Somboro AM, Hammond AS.
Siddiqui S, et al. Molecular strain typing of *Mycobacterium tuberculosis* complex in Bamako, Mali. Int J Tuberc Lung Dis 2012;16:911-6.

4. Godreuil S, Torrea G, Terru D, Chevenet F, Diagbouga S, Supply P, et al. First molecular epidemiology study of *Mycobacterium tuberculosis* in Burkina Faso. J Clin Microbiol 2007;45:921-7.

5. van Soolingen D, de Haas PE, Hermans PW, Groenen PM, van Embden JD. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of *Mycobacterium tuberculosis*. J Clin Microbiol 1993;31:1987-95.

6. National Institute of Statistics. 4th General Census of Population and Habitat in Mali-R.G.P.H. 2009, Provisional Results; 2009.

7. Ministry of Health and Public Hygiene. National Division of Health - National Tuberculosis Program, Progress Reports 2013, Bamako-Mali 2014.

8. Ministry of Health. National Division of Health - National Tuberculosis Program, Progress Reports 2009, Bamako-Mali 2010. p. 25.

9. CDC. TB Drug-Susceptibility Testing: Expert Panel Meeting, Summary Report, 12-13 December, 2007. Atlanta, GA. Available from: https://www.aphl.org/programs/infectious_disease/tuberculosis/Documents/ID_2007Dec_TB-DST-Report.pdf. [Last accessed on 2017 Oct 11].

10. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuiper S, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol 1997;35:907-14.

11. Kremer K, Arnold C, Cataldi A, Gutiérrez MC, Haas WH, Panaiotov S, et al. Discriminatory power and reproducibility of novel DNA typing methods for *Mycobacterium tuberculosis* complex strains. J Clin Microbiol 2005;43:5628-38.

12. Zhao M, Liu Y, Liu K, Wan K, Lv Z, Tu S, et al. Genetic Diversity and Drug Resistance of 133 *Mycobacterium tuberculosis* Isolates from Jiangxi Province, China. Mol Biol 2016;5:2. Available from: https://www.omicsonline.org/peer-reviewed/genetic-diversity-and-drug-resistance-of-133-mycobacteriumtuberculosis-isolates-from-jiangxi-province-china-68984.html. [Last accessed on 2017 Oct 11].

13. Diallo M, Diarra B, Sanogo M, Togo AC, Somboro AM, Diarlo MH, et al. Molecular identification of *Mycobacterium bovis* from cattle and human host in Mali: Expanded genetic diversity. BMC Vet Res 2016;12:145.

14. Gehre F, Otu J, Kendall L, Forson A, Kwara A, Kudzawu S, et al. The emerging threat of pre-extensively drug-resistant tuberculosis in West Africa: Preparing for large-scale tuberculosis research and drug resistance surveillance. BMC Med 2016;14:160.

15. Affolabi D, Anyo G, Falihan F, Sanoussi N, Shamputa IC, Rigouts L, et al. First molecular epidemiological study of tuberculosis in benin. Int J Tuberc Lung Dis 2009;13:317-22.

16. Niobe-Eyangoh SN, Kuaban C, Sorlin P, Cunin P, Thonnon J, Sola C, et al. Genetic biodiversity of *Mycobacterium tuberculosis* complex strains from patients with pulmonary tuberculosis in Cameroon. J Clin Microbiol 2003;41:2547-53.

17. Ani A, Bruvik T, Okoh Y, Agaba P, Aghaji O, Idoko J, et al. Genetic diversity of *Mycobacterium tuberculosis* complex in Jos, Nigeria. BMC Infect Dis 2010;10:189.

18. Diarra B, Siddiqui S, Sogoba D, Traore B, Maiga M, Washington J, et al. *Mycobacterium tuberculosis* Beijing Strain, Bamako, Mali. Emerg Infect Dis 2010;16:362-3.

19. Winglee K, Manson McGuire A, Maiga M, Abeel T, Shea T, Desjardins CA, et al. Whole genome sequencing of *Mycobacterium africanum* strains from Mali provides insights into the mechanisms of geographic restriction. PLoS Negl Trop Dis 2016;10:e0004332.

20. Diarra B, Cissé AB, Kodio O, Sanogo M, Baya B, Togo AC, et al. Screening new tuberculosis patients in Mali for rifampicin resistance at 2 months. Int J Mycobacteriol 2016;5 Suppl 1:S42-3.