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## Identification of mycobacteria and other acid fast organisms associated with pulmonary disease

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### ABSTRACT

**Objective:** To identify *Mycobacterium tuberculosis* (*M. tuberculosis*) and other acid fast organisms isolated from sputum of HIV positive adult patients with pulmonary disease in Jos, Nigeria. **Methods:** Acid fast organisms isolated from 80 acid fast bacilli (AFB) positive sputa of HIV positive adult patients suspected for tuberculosis in Jos, Nigeria were identified for members of *M. tuberculosis* complex (*M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti*, *Mycobacterium microti* and *Mycobacterium caprae*) by use of spoligotyping, Multiplex Gen Probe, Hain genotype assay and gene sequencing for spoligotype negative isolates. **Results:** Seven different spoligotypes of *M. tuberculosis* complex were identified from 70/80 (87.5%) total number of isolates. *Mycobacterium kansasii* (1), *Mycobacterium delvalii* (1) *Nocardia* species (1) and *Tsukamurella* species (2) were detected from 5/10 spoligotype negative isolates. **Conclusions:** Although *M. tuberculosis* is the dominant AFB associated with chronic pulmonary disease in Jos, Nigeria, other clinically relevant mycobacteria were also observed in the study. This suggests that other AFB positive microorganisms associated with tuberculosis-like symptoms might be misdiagnosed and incorrectly treated as *M. tuberculosis*. It is therefore necessary for laboratories in tuberculosis high burden countries to step up diagnostic procedures beyond routine smear microscopy.

## 1. Introduction

*Mycobacterium tuberculosis* (*M. tuberculosis*) is a pathogenic species of the genus Mycobacteriaceae and the agent of human classical tuberculosis (TB). The less virulent nontuberculous mycobacteria (NTM) found in environments such as dust and running surface waters[1–3] are morphologically indistinguishable from *M. tuberculosis*. Although not transmissible from human to human[4], NTM can cause opportunistic infection capable of multifocal organ involvement in humans, and more frequently chronic lung diseases[2,5,6]. Infection of the lungs may be similar

to classical TB but more difficult to treat and if necessary, prolonged treatment periods may be required[1,6,7]. HIV positive and severely immunocompromised persons are at high risk due to very low CD4 counts[8–11]. The lack of sensitive identification methods in most clinical laboratories may predispose to misdiagnosis of NTM disease for tuberculosis especially in resource limited settings that rely only on acid fast bacilli (AFB) smear microscopy for TB diagnosis. Although NTM have been associated with primary disease in severe immunodeficiency conditions, it could also constitute a secondary infection in active TB or after TB therapy[12,13]. It is therefore necessary to carry out comprehensive clinical and radiological investigations in infected persons, to understand the pathological role of NTM when isolated. Establishment of referral centers including expert physicians in NTM treatment and management has

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been recommended.

Published studies on *Mycobacterium* infections are scarce in Nigeria in spite of high burden of HIV and TB and the prevalence of atypical mycobacteria associated with pulmonary disease is not known. Reports from other countries have demonstrated that atypical mycobacterial infections are associated with HIV positive persons, other immunocompromised patients and transplant receivers<sup>[10]</sup>.

Conventional methods<sup>[14,15]</sup> for identification of *Mycobacterium* species are time consuming and often not specifically conclusive in species identification, while the newer biochemical (high performance liquid chromatography) and some of the highly specific molecular methods<sup>[16,17]</sup> are not cost effective for use in routine clinical laboratories. Spoligotyping<sup>[18]</sup>, a simple PCR based method distinguishes members of *M. tuberculosis* complex in clinical specimens or culture. The procedure, though not cost effective for routine use, has been widely applied in molecular epidemiology and identification of *M. tuberculosis* complex.

We identified AFB isolated from sputa in Jos Nigeria, where smear microscopy has been the most widely used laboratory method for TB diagnosis. The study examined 80 consecutive isolates from cases of pulmonary tuberculosis.

## 2. Materials and methods

### 2.1. Ethical consideration

The study which was respectively approved by the Ethical Committee of the Jos University Teaching Hospital and the Plateau State Hospital Jos, Nigeria, was descriptive of a bacterial collection and contained no material of human origin. Personal data were removed from all bacterial cultures to protect the anonymity of the patients. Ethical clearance was granted with no requirement for patient informed consent.

### 2.2. Study population

Eighty AFB positive isolates from 94 AFB positive sputa were identified by spoligotyping, GenProbe, Hain genotype and 16s ribosomal DNA gene sequencing. The strains were isolated during January 2008 to December 2009 from 790 total number of HIV patients suspected for tuberculosis in Jos, Nigeria.

### 2.3. Collection of data and specimens

Sputum specimens were collected in 1 mL solution of 1% cetylpyridinium chloride (CPC) with 2% sodium chloride and processed by standard methods<sup>[9,10]</sup> for culture on Lowenstein Jensen (LJ) medium. AFB smear microscopy was used for preliminary identification of suspect isolates. AFB positive cultures on LJ slants were subcultured and

preserved at  $-20^{\circ}\text{C}$  and subsequently shipped to NIH HIV/TB Centre, SEREFO, University of Bamako, Mali for spoligotyping and Multiplex GeneProbe. Spoligotyping was performed as described by Kermerbeek *et al.*<sup>[13]</sup>. Unidentified species were sent to the Norwegian Institute of Public Health Oslo for sequencing.

## 3. Results

Seventy of the 80 (88%) total number of isolates were *M. tuberculosis* complex spoligotypes; Latin America Mediterranean Family (LAM) (75.6%), T (10%), Haarlem (4.3%), *Mycobacterium africanum* (*M. africanum*) (2.9%), EAI (5.7%), F (1.4%) (Table 1 and 2). Only one [*Mycobacterium kansasii* (*M. kansasii*)] of the 10 spoligotype negative isolates was identified by geneprobe, 4 others *i.e.* *Mycobacterium duvalii* (*M. duvalii*) (1), *Nocardia asteroides* (1) and *Tsukamurella* species (2) were detected by 16s rRNA gene sequencing while 5/10 isolates were lost to contamination.

These results illustrate the importance of further investigation of AFB cases to exclude other mycobacteria/non mycobacterial microorganisms, especially in immunosuppressed patients suspected of having tuberculosis.

**Table 1**

Genus *Actinomycetes* isolated from sputa of pulmonary disease cases in Jos, Nigeria (n=80) [n (%)].

Genus	Frequency of isolates
<i>M. tuberculosis</i> complex	70 (87.5)
NTM	2 (2.5)
<i>Nocardia</i> spp.	1 (1.2)
<i>Tsukamurella</i> spp.	2 (2.5)
Total	75 (93.7)*

\*Five isolates were lost to contamination.

**Table 2**

Spoligotypes of *M. tuberculosis* complex isolated from Jos, Nigeria [n (%)].

MTB Family	Frequency
LAM 10	47 (67.0)
LAM 8	6 (8.6)
Haarlem	3 (4.3)
EAI	4 (5.7)
F	1 (1.4)
<i>M. africanum</i>	2 (2.9)
T	7 (10.0)
Total	70 (99.9)

## 4. Discussion

The detection of 88.5% *M. tuberculosis* complex by spoligotyping confirms that *M. tuberculosis* is the major cause of chronic pulmonary disease in Jos Nigeria and that the use of smear microscopy for prompt and presumptive diagnosis of *M. tuberculosis* remains an effective and

relevant tool especially in a resource limited setting lacking the more sensitive technological implements for more accurate and rapid diagnosis. The findings in this study agree with others in some countries where a declining incidences of tuberculosis have been reported following the practice of the directly observed treatment short course (DOTS)[19–22]. However, the emergence of drug resistance TB or the non eradication of AFB after successful completion of therapy with first line anti-tuberculosis drugs remains a concern.

The prevalence of 10/80 (12%) AFB positive and spoligotype negative isolates in this study calls to question the position of some of the cases that failed eradication with consistent acid fast positive smears after completion of treatment with first line anti-tuberculosis drugs. The detection of *M. kansasii* (1), *M. duvalii* (1), *Nocardia* spp. (1) and *Tsukamurella* spp. (2) from the 5 available isolates may not be unrelated to such cases. The pathogenic relevance of the isolates could not be explained from the available data in this study even though all five isolates were from sputa of new cases which apparently qualified the patients for recruitment under the DOTS TB treatment program. *M. kansasii* could be clinically relevant as it has been known to cause tuberculosis-like pulmonary disease in humans[2,23,24]. *Nocardi* spp. and *Tsukamurella* spp. have also been associated with pulmonary disease in humans[25–27]. There are scare reports associating *M. duvalii* with human infection although it has been reported to have some antigenic relatedness with *M. leprae*[28] and also was reported in HIV patient in India[12,29–32]. All three genera (*Mycobacterium*, *Nocardia*, *Tsukamurella*) belong to the same Family Actinomycetales with mycolic acid cell walls[26,27]. Further studies are intended to ascertain the followup treatment outcome of NTM isolates in cases treated with conventional anti-TB regimen in Jos Nigeria.

Only 94 of 790 (12%) total number of patients suspected for tuberculosis had AFB positive smear sputa. This is less than 25% estimated prevalence of TB in HIV positive cases in Nigeria. It is possible that some of the patients were unable to expectorate detectable levels of bacilli in sputa due to HIV immunosuppression. HIV and TB endemic countries need to step up laboratory diagnostic facilities to include more sensitive detection methods such as the nucleic acid amplification test (NAAT)[33] to enhance effective detection and treatment of NTM as well as other non mycobacteria pulmonary diseases. This would prevent unnecessary rise in drug resistant mycobacteria species.

The concept which suggests that non specific cross immunity develops due to latent TB against the atypical mycobacteria especially in *M. tuberculosis* endemic countries[7] may not significantly apply in HIV/TB endemic communities like Nigeria.

The dominance of LAM 10 Family of *M. tuberculosis* in this study and a previous study[34] needs to be investigated further to establish the transmission pattern of tuberculosis in Jos. Although LAM is generally reported in other West African countries[35–39], the unique homogeneity of LAM 10 seen in Nigeria has not been reported elsewhere. We have

previously suggested that the dominance of LAM family in Nigeria and West Africa may be a result of the historic interactions between West Africa and South America of which the Nigerian sea coasts served as major export route[22].

The limitations of the study included the inability to define the clinical relevance of other AFB isolated. However, the results illustrate the importance of investigating for NTMs and other non mycobacterial AFB in clinical specimens (sputa) especially in immunosuppressed patients. Such organisms may colonize the airways and cause life threatening diseases. Precise identification of some genera and species requires advanced methodologies which are not readily available in several high TB burden countries.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### References

- [1] Griffith DE, Aksamit T, Brown-Elliot BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007; **175**: 367–416.
- [2] National Jewish Health. Lung line 1–800–222–lung. 2009.
- [3] Berger E, Batra P, Ralston J, Sanchez MR, Franks AG. Atypical mycobacteria infection in an immunocompromised patient. *Dermatol Online J* 2010; **16**: 21.
- [4] Scheinfeld NS, Elston DK. Atypical mycobacterial diseases. 2011. [Online] Available from: <http://emedicine.medscape.com/article/1105570-overview> [Accessed on 20 April, 2011]
- [5] Marras TK, Chedore P, Ying AM, Jamieson F. Isolation prevalence of pulmonary non-tuberculous mycobacteria in Ontario 1997–2003. *Thorax* 2007; **62**: 661–666.
- [6] Paul S, Kenneth NO. Pulmonary nontuberculous mycobacterial disease: new insights into risk factors for susceptibility, epidemiology, and approaches to management in immunocompetent and immunocompromised patients. *Curr Infet Dis Rep* 2010; **12**: 198–203.
- [7] Cook JL. Nontuberculous mycobacteria: opportunistic

- environmental pathogens for predisposed hosts. *Br Med Bull* 2010; **96**: 45–59.
- [8] Ratanasuwan W, Tahasathit W, Chenarom V, Suwanagool S, Anekthananont T, Jearanaisilavong J, et al. Infection due to non tuberculous *Mycobacterium* other than MAC in AIDS patient at Siriraj hospital during 1998–2000: saprophyte versus pathogen. *J Med Ass Thai* 2005; **85**: 886–893.
- [9] Falkinham JO. Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. *Emerg Infect Dis* 2011; **17**: 419–424.
- [10] Singh S, Gopinath K, Shahdad S, Kaur M, Singh B, Sharma P. Nontuberculous mycobacterial infection detected in Indian AIDS patients by a novel set of ESAT–6 polymerase chain reaction primers. *Jpn J Infect Dis* 2007; **60**: 14–18.
- [11] Gopinath K, Singh S. Non tuberculous mycobacteria in TB–endemic countries: are we neglecting a danger? *PLoS Negl Trop Dis* 2010; **4**(4): e615.
- [12] Gopinath K, Singh S. Multiplex PCR assay for simultaneous detection and differentiation of *Mycobacterium tuberculosis*, *Mycobacterium avium* complexes and other mycobacterial species directly from clinical specimens. *J Appl Microbiol* 2009; **107**: 425–435.
- [13] Miguez–Burbano MJ, Flores M, Ashkin D, Rodriguez A, Granada AM, Quintero N, et al. Non–tuberculous mycobacteria disease as a cause of hospitalization in HIV infected subjects. *Int J Infect Dis* 2006; **10**: 47–55.
- [14] World Health Organization. *Stop TB partnership retooling task force, stop TB partnership new diagnostics working group. New laboratory diagnostic tools for tuberculosis control*. Geneva: WHO; 2009. [Online] Available from: <http://www.stoptb.org/retooling>. [Accessed on 18 May, 2011]
- [15] World Health Organization. *Policy guidance on drug susceptibility testing (DST) of second–line anti–tuberculosis drugs*. Geneva: WHO; 2008, p. 392. [Online] Available from: <http://www.who.int/tb/dots/laboratory/policy/en/print.html>. [Accessed on 20 May, 2011]
- [16] Zoheira D, Didier R, Mamadou D, Michel D. A single–step sequencing method for the identification of *Mycobacterium tuberculosis* complex species. *PLoS Negl Trop Dis* 2008; **2**: e253.
- [17] Wilson ML. Recent advances in the laboratory detection of *Mycobacterium tuberculosis* complex and drug resistance. *Clin Infect Dis* 2011; **52**: 1350–1355.
- [18] Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol* 1997; **35**: 907–914.
- [19] Subramani R, Radhakrishna S, Frieden TR, Kolappan C, Gopi PG, Santha T, et al. Rapid decline in prevalence of pulmonary tuberculosis after DOTS implementation in a rural area of South India. *Int J Tuberc Lung Dis* 2008; **12**: 916–920.
- [20] Progress against tuberculosis. The living proof project. Global Health Group at the University of California, San Francisco; 2009. [Online] Available from: <http://www.livingproofproject.org>. [Accessed on 28 June, 2011]
- [21] TB profile in khazastan USAID Kazakhstan. [Online] Available from: [www.usaid.gov](http://www.usaid.gov). 2009. [Accessed on 10 May, 2011]
- [22] Marica C, Didilescu C, Galie N, Chiotan D, Zellwege JP, Sotgiu G et al. Reversing the tuberculosis upwards trend: a success story in Romania. *Eur Respir J* 2009; **33**: 168–170.
- [23] Griffith DE. Respiratory infections. *Curr Opin Infect Dis* 2010; **23**: 185–190.
- [24] Santin M, Dorca J, Alcaide F, Gonzalez L, Casas S, Lopez M, et al. Long–term relapses after 12–month treatment for *Mycobacterium kansasii* lung disease. *Eur Respir J* 2009; **33**: 148–152.
- [25] Sakar MM, Gopinath K, Singh R, Singh S. *In vitro* antimicrobial drug susceptibility testing of non tubercular mycobacteria by tetrazolium microplate assay. *Ann Clin Microbiol Antimicrob* 2008; **7**: 15.
- [26] Mehta YB, Goswami R, Bhanot N, Mehta Z, Simonelli P. *Tsukamurella* infection: a rare cause of community–acquired pneumonia. *Am J Med Sci* 2011; **341**: 500–503.
- [27] Ménard A, Degrange S, Peuchant O, Nguyen TD, Dromer C, Maugein J. *Tsukamurella tyrosinosolvens*–an unusual report of bacteremic pneumonia after lung transplantation. *Ann Clin Microbiol Antimicrob* 2009; **8**: 30.
- [28] Shepard CC, Van Landingham R, Walker L. Immunity to *Mycobacterium leprae* infections in mice stimulated by *M. leprae*, BCG and graft–versus–host reactions. *Infect Immun* 1976; **14**: 919–928.
- [29] Dailloux M, Abalain ML, Laurain C, Lebrun L, Loos–Ayav C, Lozniewski A, et al. Respiratory infections associated with nontuberculous mycobacteria in non–HIV patients. *Eur Respir J* 2006; **28**: 1211–1215.
- [30] Li WF, Li ZN, Chen LR, Gong XJ. Synthesis and structural analysis of mono–dodecanoic acid esters of ginsenoside M1. *Chin J Nat Med* 2011; **9**(3): 199–203.
- [31] Wen XA, Liu J, Zhang LY, Ni PZ, Sun HB. Synthesis and biological evaluation of arjunolic acid, bayogenin, hederagonic acid and 4–epi–hederagonic acid as glycogen phosphorylase inhibitors. *Chin J Nat Med* 2010; **8**(6): 441–448.
- [32] Sun ZH, Zhang CF, Zhang M. A new benzoic acid derivative from *Eclipta prostrata*. *Chin J Nat Med* 2010; **8**(4): 244–246.
- [33] Lucian JD, Laurence H, William W, Henry M, Adithya C, Charles H, et al. Nucleic acid amplification tests for diagnosis of smear–negative TB in a high HIV–prevalence setting: a prospective cohort study. *PLoS One* 2011; **6**: e16321.
- [34] Agatha A, Torbjørn B, Okoh Y, Agaba P, Agbaji O, Idoko J, et al. Genetic diversity of *Mycobacterium tuberculosis* complex in Jos, Nigeria. *BMC Infect Dis* 2010; **10**: 189.
- [35] Ramona G, Solomon G, Jenny S, Paulo R, Raffaella C, Fabio R, et al. The Guinea–Bissau family of *Mycobacterium tuberculosis* complex revisited. *PLoS One* 2011; **6**: e18601.
- [36] Affolabi D, Anyo G, Faihun F, Sanoussi N, Shamputa IC, Rigouts L, et al. First molecular epidemiological study of tuberculosis in Benin. *Int J Tuberc Lung Dis* 2009; **13**: 317–322.
- [37] 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**: 2547–2553.
- [38] Easterbrook PJ, Gibson A, Murad S, Lamprecht D, Ives N, Ferguson A, et al. High rates of clustering of strains causing tuberculosis in Harare, Zimbabwe: a molecular epidemiological study. *J Clin Microbiol* 2004; **42**: 4536–4544.
- [39] Eldholm V, Matee M, Mfinanga S, Heun M, Dahle URA. First insight into the genetic diversity of *Mycobacterium tuberculosis* in Dar Es Salaam, Tanzania, assessed by spoligotyping. *BMC Microbiol* 2006; **6**: 76.