Indonesian Journal of
Tropical and Infectious Disease
Vol. 3. No. 1 January–March 2012

Research Report

MYCOBACTERIA AND OTHER ACID FAST ORGANISMS ASSOCIATED WITH PULMONARY DISEASE IN JOS, NIGERIA PULMONARY DISEASE AND ACID FAST ORGANISMS

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ABSTRACT

Objective: *Acid fast bacilli (AFB) for sputum smear microscopy is the affordable method used for prompt diagnosis of tuberculosis in Nigeria despite its lack of specificity and limited sensitivity. The study aims to identify Mycobacterium tuberculosis and other acid fast organisms isolated from sputum of of HIV positive adult patients with pulmonary disease in Jos, Nigeria.* ***Methods:*** *Acid fast organisms isolated from 80 AFB positive sputa of HIV positive adult patients suspected for tuberculosis in Jos, Nigeria were identified for members of M. tuberculosis Complex (M tuberculosis, M bovis, M africanum, M canetti M. microti and M. caprae) by use of spoligootyping, 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. M. kansasii (1), M. dulvalii (1) Nocardia species (1) and Tsukamurella species (2) were detected from 5/10 spoligotype negative isolates.* ***Conclusion and Recommendation:*** *Although M. tuberculosis is the dominant AFB associated with chronic pulmonary disease in Jos, Nigeria, other clinically relevant mycobacteria were observed in the study. This suggests that other AFB positive microorganisms associated with tuberculosis -like symptoms could be misdiagnosed and incorrectly treated as M. tuberculosis. It is therefore necessary for laboratories in TB high burden countries to step up diagnostic procedures beyond routine smear microscopy.*

Key words: *Acid fast bacilli (AFB) Mycobacteria tuberculosis, Other Mycobacteria species*

INTRODUCTION

Mycobacterium tuberculosis is a pathogenic species of the genus Mycobacteriaceae and the agent of human classical tuberculosis. The less virulent Non Tuberculous 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, NTMs cause opportunistic infection capable of multifocal organ involvement in humans, and more frequently chronic lung diseases.[4-5,2] Infection of the lungs may be similar to classical tuberculosis but more difficult to treat and if necessary, prolonged treatment periods may be required.[6–7] HIV positive and severely immunocompromised persons are at high risk due to very low CD4 counts.[8–10] 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 AFB smear microscopy for TB diagnosis. Although NTMs have been associated with primary disease in severe immunodeficiency conditions, it could also constitute a secondary infection in active TB or after TB therapy.[11] It is therefore necessary to carryout 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 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.[12–13]

Conventional methods[14-16] 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[17-19] are not cost effective for use in routine clinical laboratories. Spoligotyping,[20] 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 acid fast bacilli 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.

MATERIALS AND METHODS

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.

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.

Sputum specimens were collected in 1ml solution of 1% cetyl pyridinum chloride (CPC) with 2% sodium chloride and processed for culture on Lowenstein Jensen (LJ) medium.[8] AFB smear microscopy was used for preliminary identification of suspect isolates. AFB positive cultures on LJ slants were subcultured and preserved at -20° C and subsequently shipped to SEEFO NIH TB/imunology Laboratory Mali for spoligotyping and Multiplex GeneProbe. Spoligotyping was performed as described by Kermerbeek et al.[20] Unidentified species were sent to the Norwegian Institute of Public Health Oslo for sequencing.

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%), *M. africanum* (2.9%) EAI (5.7%), F (1.4%). Only one (M. *kansasii*) of the 10 spoligotype negative isolates were identified by geneprobe, 4 others; *M. duvalii* (1), *Norcardia asteroids* (1) and Tsukamurella species (2) were detected by 16s rRNA by 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

	No of isolates	%
M. tuberculosis	70	87.5
NTM	2	2.5
Nocardia spp	1	1.2
Tsukamurella 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

MTB Family	Number	%
LAM 10	47	67
LAM 8	6	8.6
HAARLEM	3	4.3
EAI	4	5.7
F	1	1.4
M	2	2.9
T	7	10
Total	70	99.9

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 agrees 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).[21-22] However, the emergence of drug resistance TB or the non eradication of acid fast bacilli 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.[8,25-26] There are scare reports associating *M. duvalli* with human infection although it has been reported to have some antigenic relatedness with *M. leprae*[27] and also was reported in HIV patient in India.[28] All three genera (Mycobacteria, Nocardia, Tsukamurella) belong to the same Family Actinomycetales with mycolic acid cell walls.[29-30] 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) to enable 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[10] may not significantly apply in HIV/TB endemic communities like Nigeria.

The dominance of LAM 10 Family of *M tuberculosis* in this and a previous study[31] needs to be investigated further to establish the transmission pattern of tuberculosis in Jos. Although LAM is generally reported in other West African countries,[32-34] 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.[31]

The limitations of the study included the Inability to define the clinical relevance of other acid fast bacilli isolated. However, the results illustrate the importance of investigating for NTMs and other non Mycobacatrial 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.

ACKNOWLEDGEMENT

Part of this study was accomplished through fellowships granted by the West African Health Organization (WAHO) and the Norwegian Institute of Public Health (NIPH), Oslo. WAHO and NIPH have no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. No additional external funding received for this study. We acknowledge the skilled technical staff of Department of Bacteriology and Immunology, NIPH.

Conflict of Interest: None

Author Contributions

AEA and URD conceived and designed the study, CL and YF did the pre analytical processing of specimens and data arrangement, URD did the gene sequencing while AEA, BD, URD, and SM performed the other assays and analyzed the data. AEA, URD and JI wrote the report which was reviewed and approved by all authors.

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