Prevalence and Risk Factors for Pulmonary Mycobacteriosis in Lagos, Nigeria

T. Y. Raheem[1,2*], B. A. Iwalokun[2], A. Oluwadun[3], O. A. Adesesan[4], N. Tochukwu[4] and M. Nshiogu[4]

[1]*Clinical Diagnostic Laboratory, Nigerian Institute of Medical Research, Yaba-Lagos, Nigeria.*
[2]*Department of Molecular Biology Biotechnology, Nigerian Institute of Medical Research, Yaba-Lagos, Nigeria.*
[3]*Department of Medical Microbiology and Parasitology, Olabisi Onabanjo College of Health Sciences, Ago-Iwoye, Ogun State, Nigeria.*
[4]*Centre for TB Research, Nigerian Institute of Medical Research, Yaba-Lagos, Nigeria.*

Original Research Article

ABSTRACT

Background: Pulmonary mycobacteriosis has been documented in HIV-infected, diabetics, asthmatics, smokers and alcoholics and its progression and severity are affected by these risk factors. Inappropriate diagnosis of mycobacteriosis could lead to inappropriate treatment with anti-tuberculosis drugs.
Methods: This cross-sectional, prospective study was conducted in patients with TB-like diseases attending six DOTs centres in Lagos, Nigeria, from May 2012 to October 2016. Participants' informed consent was obtained, structured questionnaires administered to obtain socio-demographic and co-morbid data. Sputum samples collected and processed for microscopy and

_____

*Corresponding author: E-mail: tyraheem@nimr.gov.ng;

culture using Lowenstein-Jensen medium with or without pyruvate and MGIT 960 liquid medium. Mycobacteria were identified using MPT64 immunochromatographic, biochemical and molecular methods. This study investigated the presence and prevalence of mycobacteriosis in the participants and assessed the risk factors for the mycobacterial infections.

Results: Of the 1,020 participants, 339 (33.2%) had mycobacteriosis of which 33 (9.7%) were caused by *Non-Tuberculosis Mycobacteria (NTM)* and 306 (90.3%) caused by *Mycobacterium tuberculosis complex (MTBC)*. Of the isolated 306 *MTBC,* 247 (80.7%) were *M. tuberculosis, 28 (9.2%) were M. africanum*, 23 (7.5%) were *M. bovis* while 8(2.6%) were *M. ulcerans* [P < 0.0005]. The 33 *NTM* showed 11 (33.3%), 20 (60.6%) had HIV, 8*(24.2%) M. fortuitum*, 2 (6.1%) *M. abscessus*, 2 (6.1%) *M. scrofulacium*, 6 (18.2%) *M. kansasii*, 4 (12.1%) *M. megateriense* and 11 (33.3%) *Mycobacterium avium complex (MAC)*. Sequence analysis of the 16s rRNA of the 11 *MAC* showed 3 (27.3%) *M. avium*, 5(45.5%) *M. intracellulare*, 2(18.2%) *M. colombiense* and 1(9.1%) *M. velneri*. *M. fortuitum* and *MAC* were significantly (P<0.05) associated with HIV infection, while only *M. fortuitum* relate strongly with diabetes (P <0.05).

Conclusion: The study showed that mycobacteriosis is caused by different species of *MTBC* and *NTM*. Relatively high mycobacteriosis were detected during dry season and were significantly associated with gender, age, HIV and diabetes.

ABBREVIATION

DOTs=Directly Observed Therapy Short Course

1.BACKGROUND

Mycobacteriosis *is* defined as infection caused by different species of *Mycobacteria* including Non-*Tuberculosis Mycobacteria (NTM) and Mycobacterium Tuberculosis Complex* [1, 2, 3]. *M. tuberculosis* is the commonest specie of Mycobacteria that causes pulmonary tuberculosis and it infects one third of the human world population and kills someone every 15 seconds [4,5]. In Nigeria, tuberculosis (TB) is a major public health problem. It was declared a national emergency in June 2006 after which a plan for the control of TB in Nigeria was developed [6].

Despite expansion in case finding and DOTS coverage in the last 15 years in the country, the national case detection rate of 41% is still far below the 70% national and global target. This had been attributed to limited facilities for sputum culture and mycobacterial identification in the country coupled with poor access to health facilities and health seeking behaviour of TB suspects, particularly in the rural areas [3,7]. NTM infections have been associated with the reactivation of latent TB and TB relapse or re-infection in previously cured patients [5,8]. They enhance the non-immunity effect of previous TB exposure [9,10]. This is also among the challenges faced by the global TB elimination efforts [3,11].

The need for sputum culture and mycobacterial characterisation has become very important. This is to rule-out mixed infections and Non-Tuberculous Mycobacteria (NTM) that are now on the increase in TB endemic developing countries and has out-numbered *M. tuberculosis* in incidence and prevalence in developed countries [3]. NTM which are environmental Mycobacteria found in water bodies, soil, animals and food products [8,9] are increasingly being reported as causes of infections in immunocompetent and immunocompromised patients in Africa like in many developed countries of the world. Infections caused by the species include pulmonary infection, disseminated infection, meningitis, cervical lymphadenitis and pneumonitis [8]. The immunocompromised patients for which NTM has been documented to play a role in the pathogenesis, progression and severity of pulmonary infections include HIV seropositive patients, diabetes patients, patients with asthma, chronic obstructive pulmonary disease (COPD), nodular bronchiectasis and silicosis [8]. In Nigeria, a few studies have reported the occurrence of pulmonary infections due to NTM in Lagos [10]. There is no doubt that Nigeria requires accurate characterisation of mycobacteria, rational use of first-line anti-TB regimen, improved knowledge of the role played by NTM in pulmonary and disseminated infections in Nigerian patients.

The objectives of the study were to investigate the presence and prevalence of Mycobacterial infections (mycobacteriosis) in patients

suspected of pulmonary tuberculosis and to assess the risk factors responsible for the mycobacteriosis.

2. METHODS

2.1 Study Sites

This was a multicenter study covering randomly selected six health facilities with DOTs services in Lagos.

2.2 Study Design

The study was a cross-sectional, prospective study on patients suspected of pulmonary mycobacterial infections (suspected TB patients) from May 2012 and October 2016.

2.3 Sample Size

Specimen collection: 1020 participants were enrolled and sputum samples collected from them. At enrolment, a pre-tested semi-structured questionnaire was administered per participant by a trained health worker to capture socio-demographic data such as age, gender, education, marital status and occupation. Information on tobacco smoking and alcohol intake habits as well as diagnosis or treatment for diabetes was also obtained. Each participant was then screened for HIV 1/II according to the national algorithm [6,12]. Two sputum samples-one on the spot (day 1), followed by the second samples (day 2) collected at early morning were screened microscopically for presence of acid fast bacilli (AFB) and processed for culture as described by [13,14], MGIT manual, biochemical tests, immune-chromatographic (ICT) test and line probe molecular assay method.

2.4 Data Analyses

Data obtained after questionnaire administration were double entered into Microsoft excel 2007 version and Epi Info version 6.1. They were validated for completeness and error before transfer to Statistical Package for Social Science (SPSS version 20) where analyses were done. Demographic variables such as age, sex, education, occupation, alcohol intake and clinical data such as presence of fever, cough, haemoptysis, night sweat, diabetes, and HIV were used as covariates and summarized as frequency and percentages (%) as well as mean $\pm$ standard deviation (SD). Chi square (X^2) of

Fischer Exact (when frequency (n) < 5) test was used to evaluate the relationship between NTM occurrence and the covariates. Covariates with significant odd ratio (OR) and 95% confidence interval (95%CI) in the Logistic regression analysis were entered into multivariate Logistic regression model to independent predictors of NTM infections

2.5 Sputum Culture

Sputum samples collected from patients with suspected pulmonary infections were decontaminated and digested with 2 volumes of N-acetyl-L-cystein 4% sodium hydroxide (NALC-NaOH) as described [13, 15]. This was followed by centrifugation using refrigerated centrifuge at 3000 rpm for 15 min. The concentrated sediment was then used to prepare smear on a grease-free slide for ZN acid-fast staining. Sputum smear microscopy was performed on stained concentrated sputum smears prior to culture and on stained culture isolates according to NTLCP guidelines [6]. The remaining sediment was then suspended in 1.5mL of phosphate buffered saline (PBS, pH 6.8) in a Falcon tube, covered and mixed by repeated inversion (2x). Aliquots (0.2mL each) of the homogenate were then used to inoculate Lowenstein-Jensen (LJ) slopes with and without sodium pyruvate [13] as well as 0.5mL into Mycobacteria Growth Indicator Tube 960 [15] containing oleic acid-albumin-dextrose-catalase and polymyxin-amphotericin B-nalidixic acid and trimethoprim-azlocillin. All inoculated media were incubated at 37°C. Bactec MGIT 960 vials were introduced into the Bactec MGIT 960 instrument as recommended by the manufacturer and tested either until they were found to be positive or for 6 weeks. The LJ medium with and without pyruvate slants were examined weekly for 8 weeks for the visible appearance of colonies. After confirmation of mycobacterial growth in a liquid or solid medium, the parallel cultures were read daily. On the day of detection, all positive liquid and solid media were examined by ZN staining to confirm the presence of AFB and sub-cultured onto Columbia agar with 5% sheep blood to check for contaminants. Samples that failed to show viability or turbidity at 8 weeks were regarded as negative for mycobacteria infections. *M. tuberculosis* on LJ was indicated as a slow growing ($\geq$16 days) pale cream rough dry colonies, including few ones that were granular and mucoid. Similar colonies on LJ sodium pyruvate medium were suspected to be those of *M. bovis.* Other fast (< 14 days) and slow growing yellow/orange pigmented colonies

on LJ slant were taken as non-tuberculous mycobacteria (NTM).

2.6 Identification of Isolates

Phenotypic methods such as Nitrate reduction Catalase Test, Growth on p-nitro benzoate (PNB) Medium, Tween 80 Hydrolysis test, Urease production test, MPT64 Immuno-chromatographic Assay and Hain's Line Probe Assay (LPA) for common mycobacteria (CM) and atypical mycobacteria strains (AS) were used as described by Hains Line Probe technique.

MPT64 Immuno-Chromatographic Technique (ICT) was validated with reference mycobacterial and other bacterial strains.

The 16s rRNA gene of the 11 *M. avium* complex (MAC) was amplified from the DNA sample of each isolate by PCR using primers sp1 (5'-ACCTCCTTTCTAAGGAGCACC-3') and sp2 (5'-GATGCTCGCAACCACTATCCA-3') as previously reported [16,17] The sequencing reactions were performed in 3170 Applied Biosystem sequencer. These sequences were further compared with those deposited in GenBank, using the BLAST algorithm [18] Sequences that showed 98% identity at comparison were then considered as identified species as described in previous study [19].

3. RESULTS

M. tuberculosis H37Rv used as control strain produced positive reaction with goat anti-MPT64 monoclonal antibody due to its secreted MPT64 antigen, other reference strains tested including *M. bovis* BCG Pasteur, *M. kansasii* and *E. coli* ATCC 25922 gave negative reaction.

The mean age of the 1,020 participants was 35.3 years (standard error of mean (SEM): 2.7 yr) and 164 (16%) had tertiary education (Table 1). The risk factors for MTBC infection were found to include gender [male 607 (59.5%) and female 413 (40.5%)] (AOR, 1.6, 95% confidence interval (CI): 1 – 2.6, P = 0.033), age 36 years and above (AOR, 1.6, 95% confidence interval (CI): 1 – 2.6, P = 0.033). Of the 1020 participants, 382 (37.5%) had bacterial pathogens. Non-mycobacteria (NMY) bacterial pathogens was 43 out of 382 (11.3%) of all bacterial isolates while 339 (88.7%) were identified as Mycobacteria. Of this, 33 (9.7%) were NTM and 306 (90.3%) were MTBC (Fig. 1). The analysis of the 33 *NTM*

showed 8(24.2%) *M. fortuitum*, 11 (33.3%) *M. avium* complex, 2 (6.1%) *M. abscesses*, 2 (6.1%) *M. scrofulacium*, 6 (18.2%) *M. kansasii* and 4 (12.1%) *M. megateriense* (Fig. 2) and out of which, 11 (33.3%) and 20 (60.6%) had HIV and represented previously treated cases. Among the 306 *Mycobacterium tuberculosis complex (MTBC) isolated,* 247 (80.7%) were *M. tuberculosis, 28 (9.2%) were M. africanum,* 23 (7.5%) were *M. bovis* while 8(2.6%) were *M. ulcerans* [P < 0.0005].

Sequence analysis of the amplified 16s rRNA of 11 *M. avium* complex (MAC) isolates revealed the identity of the isolates as 3 (27.3%) *M. avium*, 5(45.5%) *M. intracellulare*, 2(18.2%) *M. colombiense* and 1(9.1%) *M. velneri*.

M. fortuitum and *M. avium* complex (MAC) were significantly (P<0.05) associated with HIV infection, while only *M. fortuitum* relate strongly with diabetes (P <0.05). On the whole, 62.5% of the HIV seropositive patients and 57.1% of those with diabetes had NTM infections (P<0.05). Among the species of NTM isolated, *M. fortuitum* and *M. avium* complex (MAC) were significantly (P<0.05) associated with HIV infection, while only *M. fortuitum* relate strongly with diabetes (P <0.05).

Of the 339 analysed, 115 (33.9%) engaged in trading, 134 (39.5%) were artisans and 90 (26.6%) were unemployed (Table 2). The number of patients living with diabetes was 59 (17.4%), while 18 (5.3%) of the patients were HIV seropositive. Alcohol intake and tobacco smoking were documented in 74 (21.8%) and 81 (23.9%) patients respectively. Investigation of treatment history showed 12.2% of the patients to represent previously treated TB cases. The percentage of *MTBC* patients with diabetes was 4.2%, while 11.4% were previously treated TB cases. On the whole, variables such as age, education, occurrence of diabetes and HIV sero-positivity were found to influence variation in the distribution of mycobacterial and non-mycobacterial infections associated with clinical symptoms of tuberculosis in the studied patients. Cough at a rate of 50 – 100% was the most frequent symptom reported (Table 3), while haemoptysis was the least in patients infected with MAC (18.2%) and *M. abcessus* (50%). The two patients infected with *M. scrofulaceum* reported weight loss and night sweat, On the whole, 90.9% of the NTM infected patients reported at least one of these symptoms. The months with high occurrence of NTM infections
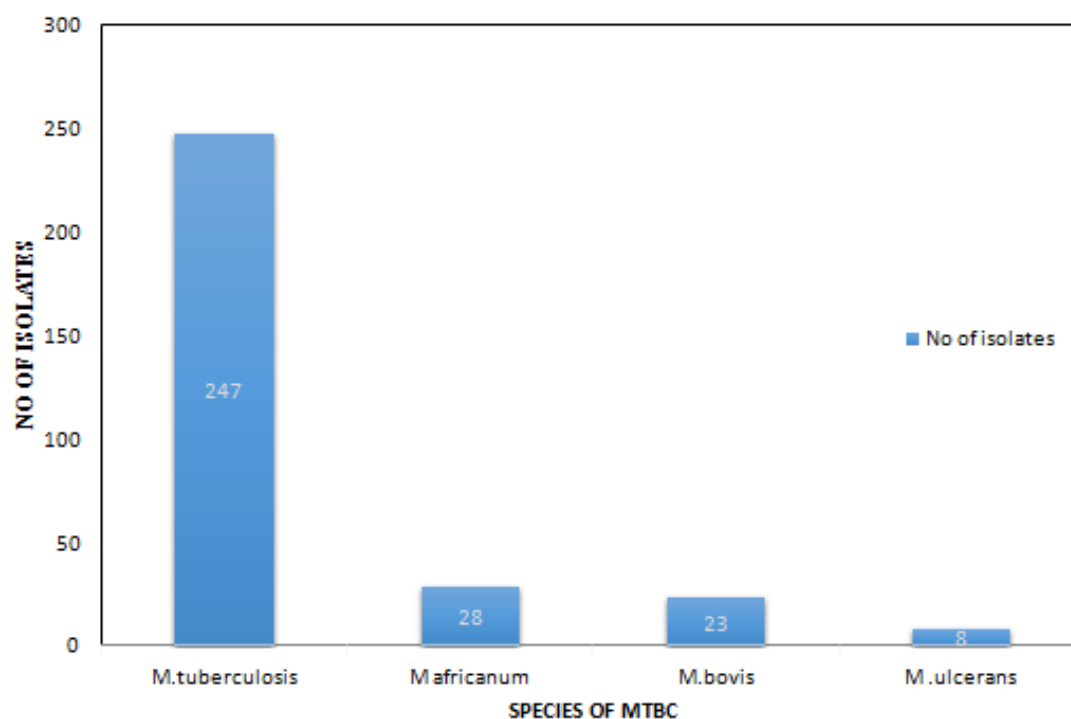
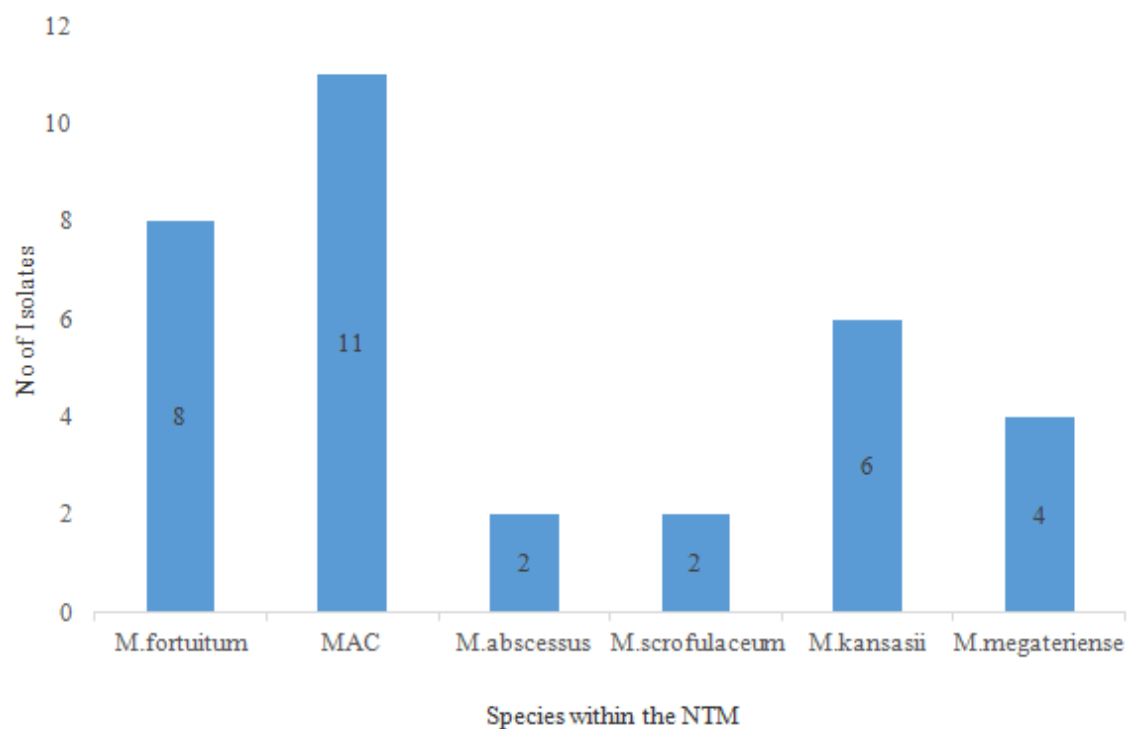**Fig. 1. Distribution of the species of MTBC isolated from the participants [p< 0.001]**

Fig. 2. Distribution of species of NTM isolated from the participants

Table 1. Distribution of aetiologies of suspected pulmonary tuberculosis according to socio-demographic, behavioral and environmental characteristics of the patients

Characteristics	Total isolates N = 339 N (%)	MTB-C N = 306 n (%)	NTM N =33 n (%)	Non-mycobacterial infection (NMY) N = 43 (%)	P-value (x^2 or t-test)
Age group, yr, n (%) 18 – 35					
≥36	154(45.4)	140(45.7)	11 (33.3)	34 (79.1)	25.9; < 0.0001
	185(54.3)	166(54.3)	22 (66.7)	9 (20.9)	
Mean age, yr					
(mean ± SEM)	34.3+1.5	36.1+1.2	32.5 + 0.4	33.4+ 1.4	
Gender, n (%)					
Male	205(60.5)	188(61.4)	20 (60.6)	25 (58.1)	
Female	134(39.5)	118(38.6)	13(39.4)	18 (41.9)	0.41; 0.81
Education, n (%)					
Primary	122(35.9)	106(34.6)	10 (30.3)	28 (65.1)	
Secondary	167(49.3)	165(53.9)	15 (45.5)	9 (20.9)	27.8; <0.0001
Tertiary	50(14.8)	35(11.4)	8(24.2)	6 (14)	
Occupation, n (%)					
Trading	92(27.1)	87(28.4)	9 (27.3) 13 (39.4)	11 (25.6)	
Artisan	103(30.3)	86(28.1)		7 (16.3)	
Civil servants	39(11.5)	38(12.4)	3(9.1)	9 (20.9)	
Private sector worker	31 (9.1)	28 (9.2)	2 (6.1)	10 (23.3)	28.7; 0.00041
Unemployed	74(21.8)	67(21.9)	6 (18.2)	6 (14)	
Diabetic, n(%)					
Yes	45(13.3)	76(24.8)	1 (3.0)	8 (18.6)	18.7; < 0.0001
No	294(86.7)	230(75.2)	32(97.0)	35 (81.4)	
HIV seropositive, n (%)					
Yes	37 (10.9)	254(83.0)	3 (9.1)	0 (0)	
No	302(89.1)	52 (17.0)	30 (90.9)	43 (100)	7.6; 0.02
Alcohol intake (%)					
Yes	56 (16.5)	265(86.6)	7(21.2)	5 (11.6)	
No	283(83.5)	41(13.4)	26 (78.8)	38 (88.4)	12.1; 0.002
Smoking, n (%)					
Yes	61 (18)	50 (16.3)	3 (9.1)	16 (37.2)	16.4; 0.0003
No	278 (82)	256(83.7)	30 (90.9)	27 (62.8)	

Characteristics	Total isolates N = 339 N (%)	MTB-C N = 306 n (%)	NTM N =33 n (%)	Non-mycobacterial infection (NMY) N = 43 (%)	P-value (χ2 or t-test)
Treatment history, n(%)					
Newly diagnosed	297(87.6)	64(20.9)	27 (81.8)	43 (100)	10; 0.007
Previously treated	42(12.4)	242(79.1)	6 (18.2)	0 (0)	

MTB-C= Mycobacterium tuberculosis complex; NTM=Non tuberculosis mycobacteria, NMY=Non Mycobacteria

Table 2. Distribution of non-tuberculous mycobacteria species among participants with HIV and diabetes

NTM species	HIV positive (Total=40), n (%)	P value	Diabetes positive (Total = 14), n (%)	P value
M. fortuitum,	5 (12.5)	0.02	3 (21.4)	0.02
MAC	9 (22.5)	0.00001	2 (14.3)	0.34
M. abcessus	2 (5)	0.08	0 (0)	0.33
M. scrofulaceum	1 (2.5)	0.53	1 (7.1)	0.38
M. kansasii	4 (10)	0.08	2 (14.3)	0.13
M. mageritense	4 (10)	0.08	0 (0)	0.67
Total	25 (62.5)	<0.000001	8 (57.1)	0.0001

There is significant association between NTM infections and HIV and Diabetes

Table 3. Distribution of the NTM species by symptoms reported by the infected patients

| NTM species	No. of isolates	Cough, N (%)	Night sweat, n (%)	Weight loss, n (%)	Haemoptysis n (%)	Chest pain n (%)	Fever n (%)	Any symptom n (%)
M. fortuitum,	8	7 (87.5)	3 (37.5)	2 (25)	0 (0)	5 (62.5)	2 (25)	8 (100)
MAC	11	8 (72.7)	6 (54.5)	5 (45.5)	2 (18.2)	4 (36.4)	4 (36.4)	9 (81.8)
M. abcessus	2	2 (100)	0 (0)	1 (50)	1 (50)	2 (100)	2 (100)	2 (100)
M. scrofulaceum	2	1 (50)	2 (100)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)
M. kansasii	6	4(66.7)	4 (66.7)	2 (33.3)	0 (0)	2 (33.3)	1 (16.7)	5 (83.3)
M. megateriense	4	2 (50)	3 (75)	1 (25)	0 (0)	0 (0)	1 (25)	4 (100)
Total	33	22(66.7)	18(54.5)	15 (45.5)	3 (9.1)	12 (36.4)	12 (36.4)	30 (90.9)
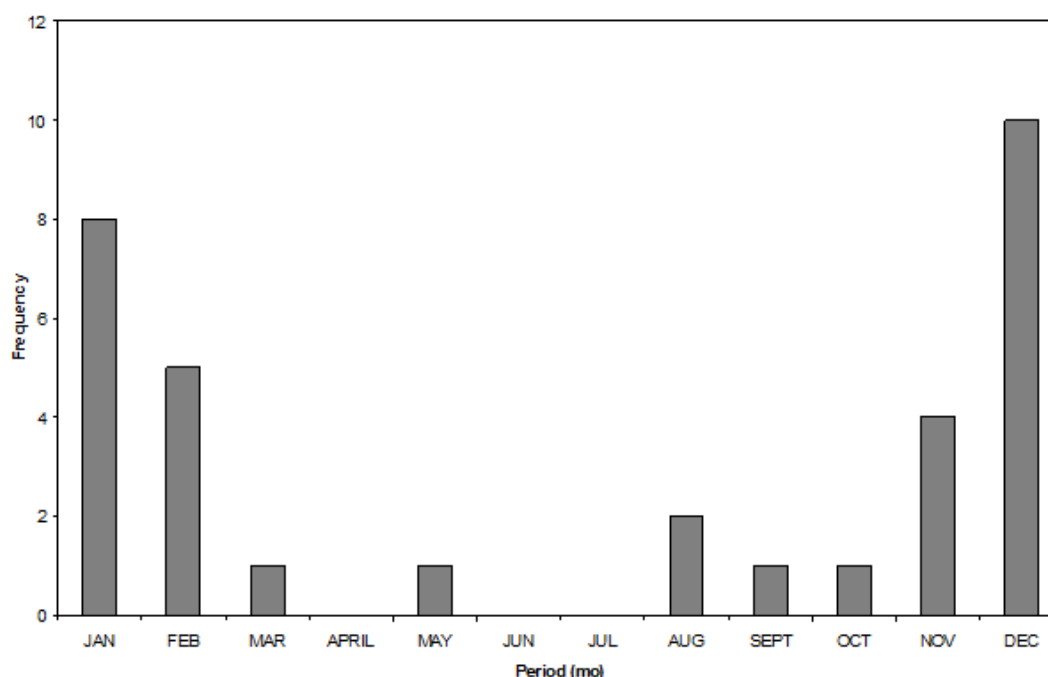
Fig. 3. Monthly occurrence of *NTM* infection among the patients with suspected tuberculosis

were found to be January (24.2%), February (12.1%) and November (15.2%) during the harmattan period. Isolates were not recovered in April, June and July at the peak of the rainy season (Fig. 3).

4. DISCUSSION

More men (59.5%) than women (40.5%) were reported in this study. This finding is similar to the report of [20] who reported a male to female ratio of 1.3:1. This result also agreed with the data reported by [21, 22]. However, the report of this study was different from those of other studies [23] where more females were reported. The higher prevalence of TB among males than females in this report has also been reported by various researchers in South-East Nigeria where PTB prevalence of 35.5% among males and 26.9% among females in South-Eastern Nigeria had earlier reported [24]. PTB prevalence of 65% and 35% among males and females respectively in Lagos had been reported [25]. The higher prevalence of PTB among males could be as a result of frequent contact with infective droplets from contaminated environment since tuberculosis is acquired through in inhalation of infectious droplets [23]. It has also been reported that males predominate among TB cases in most countries and that variation in the effect of

gender in harbouring MDR-TB could be multifactorial which could include poor knowledge about TB and "male ego" that is common with males making them seek alternative local herbs in most cases [26]. The NTM species identified in this study include 8 (24.2%) *M. fortuitum*, 2 (6.1%) *M. abscessus*, 2 (6.1%) *M. scrofulacium*, 6 (18.2%) *M. kansasii*, 4 (12.1%) *M. megateriense* and 11 (33.3%) *Mycobacterium avium complex (MAC)*. Sequence analysis of the 16s rRNA of the 11 *MAC* showed 3 (27.3%) *M. avium*, 5(45.5%) *M. intracellulare*, 2(18.2%) *M. colombiense* and 1(9.1%) *M. velneri*. The species of NTM identified in this study is similar to the Ibadan study where *M. chelonae, M. intracellulare* and *M. avium complex (M. intracellulare, M. scrofulaceum)* were also reported. This attest to the earlier report that in the setting of disease development, NTM share similar symptomatology with *M. tuberculosis* and that both groups of Mycobacteria can also not be differentiated by radiology, making accurate diagnosis of MTBC challenging at primary health care settings where culture and Mycobacterial identification facilities are lacking in the country[3,11]. Unfortunately, there is no reporting system for NTM in many developing countries including Nigeria. This is partly due to poor awareness of the clinical relevance of NTM, their

8

environmental preference and lack of evidence for person to person transmission of NTM in humans [11]. The presence of NTM in sputum specimen may lead to misdiagnosis of MTBC and inappropriate treatment with first–line anti-TB regimen (i.e. rifampicin, isoniazid, ethambutol and pyrazinamide) and second-line regimen, including injectable Aminoglycosides (e.g Amikacin or Kanamycin), Capreomycin and Fluoroquinolones [3,11,12]. It has been reported that slow-growing NTM such as *Mycobacterium avium complex (MAC) and M. kansasii* require macrolide-based regimen for case management and that NTMs have inherent resistance to the standard first-line and second-line anti TB drugs [5]. NTM infected patients are also at high risk of drug toxicities with these regimen, necessitating replacement of isoniazid with a fourth generation fluoroquinolone such as moxifloxacin [5]. The End TB Strategy, which Nigeria has also adopted, entails the reduction of TB cases by 80% and deaths by 90% by 2030 compared to 2015 and the subsequent elimination of TB by 2050 [13].

Currently in few facilities in Nigeria, mycobacteria characterization is performed by culture of smear positive sputum samples on Lowestein Jensen slope followed by biochemical tests to differentiate between mycobacteria species that constitute the MTBC complex. This study showed the need for a review of the TB treatment national guidelines which stipulates that most rapid mycobacteria positive sputum culture (of $\leq$ 2 weeks) are often regarded as contaminants and affected patients were not eligible for DOTS [6].

Age groups of the participants with tuberculosis in this study range between 15-54 years. This agreed with the report by other studies [21,23 and 27]. The reason for this is because TB usually affects young people. This account for why TB disease is said to be a disease that affect economically productive age groups.

The isolation of 90.3% *MTBC* in this study was slightly higher than the 85% strains of MTB complex reported by other studies [28]. The 9.7% mycobacteriosis due to NTM and the detection of 11 (33.3%) and 20 (60.6%) in HIV and previously treated cases implied that in HIV and in previously treated TB cases, AFB detected by sputum smear microscopy could be NTM. This could inappropriately be diagnosed as MDRTB. Therefore, there is the need for culture and characterization of the mycobacterial isolates to rule-out or confirm mycobacteriosis due to NTM in such cases. This finding also agreed with the report of [3,9,27,28] who reported similar findings in subjects with and without HIV and that *Non-Tuberculous Mycobacteria* (*NTM*) are involved in a range of diseases including pulmonary disease, hypersensitivity pneumonitis, cervical lymphadenitis, and disseminated infection and disseminated infection is generally associated with HIV infection. The prevalence of 9.7% of NTM in this study was however lower than 50% NTM reported by other Researchers among the HIV positive subjects [9]. It is also lower than the 11.6% reported by others in Lagos [25], the 13% reported in North Central part of Nigeria [26], the 15% prevalence reported [27] in subjects with and without HIV positivity and the 39% prevalence reported in Ibadan [3]. The prevalence of NTM in this study however agreed with the study of [29] who reported that NTM infections (mycobacteriosis due to NTM), vary between 4.1 to 47.0%. NTM infections have also been linked to harmattan dust exposure and to HIV co-infection; and have been reported to be a novel public health challenge which needs to be considered when planning for prevention and treatment of mycobacteriosis patients [28]. Education, occupation, smoking, alcohol intake, HIV and diabetes are confirmed to be associated with mycobacteriosis ($p < 0.05$). These results agreed with the earlier one reported by other researchers [3, 9, 28]. This finding is very important in the need for better understanding of the efficacy of the first line anti-TB treatment regimens because the responses to the anti TB regimens by mycobacteriosis caused by *NTM* are known to vary from mycobacteriosis caused by *M. tuberculosis complex* [28]. Treatment of TB patients in most sub-Saharan African countries including Nigeria, is based solely on the results of microscopic smear positivity. Patients diagnosed using sputum smear positive results alone, are indiscriminately placed on DOTS using first-line anti-TB drugs in the current TB treatment strategy. The implication of the treatment strategy based on smear microscopy results alone is that *NTM* could be inappropriately managed with first-line anti-tuberculous drug thereby possibly worsening the patient's condition and raising the risk of drug resistance.

The occurrence of 80.7% *M. tuberculosis*, 9.2% of *M. bovis,* 7.5% of *M. africanum* and 2.6%) of *M. ulcerans* of the total *MTBC* in this study agreed with the previous report that most sputum

smear positive patients are caused mainly by *M. tuberculosis* [9]. The results are also similar to 94.4% *Mycobacterium tuberculosis*, 5.3% had *Mycobacterium africanum* and 0.3% had *Mycobacterium bovis* [29]. The prevalence of 7.5% *M. bovis* reported in this study was higher than 0.3% reported by others [29]. This may be due to the fact that the study site in this study is from Lagos, in south western part of Nigeria, where the population and consumption of dairy products is higher unlike the study conducted in Zaria- North western part of Nigeria [26]. This also implied that *M. bovis* is still a common cause of pulmonary tuberculosis in the study area. The production of dairy milk and cheese from cattle locally, could be responsible possibly due to non-pasteurization of such milk. This finding is however, contrary to earlier report that *M. bovis* was once a common cause of tuberculosis, but since the introduction of pasteurized milk, it has been largely eliminated as a public health problem in developed countries [29].

5. CONCLUSIONS AND RECOMMENDAT-IONS

This study showed that mycobacteriosis can be caused by *Mycobacterium tuberculosis complex (MTBC)* and or *Non Tuberculosis Mycobacteria* (*NTM*) of which many species were detected in this study*. NTM* mycobacteriosis was associated with dry season, HIV and diabetes as risk factors. Case detection in suspected cases of pulmonary mycobacteriosis should be referred for culture and identification because only microscopy often used for DOTs programme, could be misleading and could give exaggerated data on tuberculosis, possible false impression of MDRTB and inappropriate anti-TB treatment regimen. It is recommended that capacities for TB culture and identification must be strengthened. Large scale, multi-centre, nation-wide study of mycobacteriosis is also recommended.

WHAT IS ALREADY KNOWN ON THIS TOPIC

• That mycobacteriosis is a form of opportunistic infection especially in immunocompromised

• That In dry season, respiratory illnesses are common and these include mycobacteriosis.

WHAT THIS STUDY ADDS

• Not all sputum smear positive cases should be placed on the usual anti TB regimen. It could be a case of mycobacteriosis caused by NTM and may require drugs different from the usual first-line anti TB regimen.

• Six (6) different species of NTMs were identified in this study

• Not all sputum smear positive cases are caused by *mycobacterium tuberculosis complex*. There is need to investigate mycobacteriosis due to NTMs for effective treatment regimen.

CONSENT

Samples were collected from participants who voluntarily gave informed consent and were able to submit 2 consecutive sputum samples.

ETHICAL APPROVAL

The study was also approved by Institutional review Board of the Nigerian Institute of Medical Research, Yaba, Lagos.

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wolters Kluwer, Lippincott Williams and Wilkins. Stedman's Medical Dictionary; 2012. Publisher Julie K Stegman. ISBN 978-1-60831-692-2).

2. Palomino JC, Leão SC, Ritacco V in *Tuberculosis:* From basic science to patient care 2007;406-408. Available: www.TuberculosisTextbook.com

3. Cadmus Simeon Idowu, Bassirou Diarra, Brehima raore, Mamoudou Maiga, Sophia Siddiqui, Anatole Tounkara, Olutayo

Falodun, Wole Lawal, Isaac Folorunso Adewole, Rob Murphy, Dick van Soolingen and Babafemi Nontuberculous Mycobacteria Isolated from Tuberculosis Suspects in Ibadan, Nigeria Journal of Pathogens. Taiwo; 2016;5.
Article ID 6547363,
Available:http://dx.doi.org/10.1155/2016/6547363

4. Hershberg R, Lipatov M, Small PM, Sheffer H, Niemann S: High functional diversity in Mycobacterium tuberculosis driven by genetic drift and human demography. PLoS Biology. 2008;6(12): e311.
DOI:10.1371/journal.pbio.0060311

5. World Health Organization: Global Tuberculosis Report. 2015;1-50. WHO/HTM/TB.22.

6. National Tuberculosis and Leprosy Control Programme publication: Modules for Training General Health care Workers on TB Control 4th Edition. 2011;37-98.

7. Lana Dinic, Patrick Akande, Emmanuel Oni Idigbe, Agatha Ani, Dan Onwujekwe, Oche Agbaji, Maxwell Akanbi, Rita Nwosu, Bukola Adeniyi, Maureen Wahab, Chindak Lekuk, Chioma Kunle-Ope, Nkiru Nwokoye, Phyllis Kanki Genetic Determinants of Drug-Resistant Tuberculosis among HIV-Infected Patients in Nigerian. Journal of Clinical Microbiology. 2012;50 (9):2905- 2909.

8. Griffith Chris, Pat Sturdy, Penny Brewin, Graham Bothamley, Sandra Eldridge, Adrian Martineau, Meg MacDonald, Jean Ramsay, Suresh Tibrewal, Sue Levi, Ali Zumla, Gene Feder: Educational outreach to promote screening for tuberculosis in primary care: A cluster randomised controlled trial. Lancet. 2007;369:1528–1534

9. Pokam, Benjamin T, Asuquo, Anne E: Acid-Fast Bacilli Other than Mycobacteria in Tuberculosis Patients Receiving Directly Observed Therapy Short Course in Cross River State, Nigeria. Tuberculosis Research and Treatment. 2012; 4 ArticleID301056,
DOI:10.1155/2012/301056.

10. Idigbe EO, Anyiwo CE, Onwujekwe DI. Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. Journal of Tropical Medicine and Hygiene. 1986; 89:143-148.

11. Van Halsema CL,Chihota VN, Gey van Pittius NC, Fielding KL, Lewis JJ, van Helden PD, Churchyard GJ, Grant AD: Clinical Relevance of Nontuberculosis Mycobacteria Isolated from Sputum in a Gold Mining Workforce in South Africa: An Observational, Clinical Study. Biomedical Research International; 2015. Epub.10.1155/2015/959107

12. National Tuberculosis and Leprosy Control Programme: The National Strategic Plan for Tuberculosis Control. Towards Universal Access to Prevention, Diagnosis and Treatment.2014;60-75..

13. Kent PT, Kubica GP. Public Health Mycobacteriology. A guide for the level III laboratory. US Department of Health and Human Services, Centre for Disease control, Atlanta. 1985;50-88.

14. Scott HM, Flynn JL. *Mycobacterium tuberculosis* in Chemokine Receptor 2-Deficient Mice: Influence of Dose on Disease Progression. Infection and Immunity.2002;70: 5946–5954.

15. SH Siddiqi: MGIT 960 ™ Procedure Manual-Foundation for Innovative New Diagnostics .2006;9-16.

16. Roth A, Fisher M, Hamid E, Michalke S, Ludwig W and Mauch H. Differentiation of phylogenetically related slowly growing mycobacteria based on 16S-23S rRNA gene internal transcribed spacer sequences. Journal of Clinical Microbiology. 1998;36:139-147.

17. NCBI:www.blast. ncbl.nlm.nih.gov/Blast.cgi.

18. Turenne, Christine Y, Lorelee Tschetter, Joyce Wolfe and Amin Kabani: Necessity of Quality-Controlled 16S rRNA Gene Sequence Databases: Identifying Non-Tuberculous Mycobacterium Species. Journal of Clinical Microbiology. 2001; 39(10): 3637–3648.

19. Adejumo OA, Daniel OJ, Abdur-Razzaq HA, Shogbamimo YO, Femi-Adebayo T, Adepoju VA, Adebayo BI and Sodipo OO: Trend of tuberculosis case notification and treatment outcome in Lagos State, Nigeria: a 5-year retrospective study. Transactions Royal Society of Tropical Medicine and Hygiene. 2017;111 (7):300-307.

20. WHO report: Global tuberculosis control (WHO/HTM/TB/.16). 2011;54-55.
ISBN 978 92 4 156438 0)

21. Okodua M, Ihongbe J, Esumeh F. Pulmonary Tuberculosis and Resistance Pattern to first line Anti-tuberculosis Drugs in a City of Western Nigeria. International

Journal of Basic, Applied and Innovative Research. 2012;1(2):48 – 56.

22. Raheem TY, Onubogu CC, Igbasi UT, Nwokoye N, Tochukwu N, Kunle-Ope C, Ejezie C, Omoloye R, Adesesan AA, Okoye RN, Ajayi F, Nureni A, Oba AO and Onwujekwe D. Observed Tuberculosis Treatment Outcomes among Patients with Different Human Immunodeficiency Virus Sero-status at a Health Facility in Lagos, Nigeria; Journal of Medical Laboratory Science. 2013;22(1): 3-7.

23. Itah AY, Udofia SM : Epidemiology and Endemicity of pulmonary tuberculosis (PTB) in South-Eastern Nigeria. South Asian Journal of Tropical Medicine. Public Health. 2005;36(2):317-323.

24. WHO report. Global tuberculosis control (WHO/HTM/TB/.16). 2011;54-55. ISBN 978 92 4 156438 0).

25. Idigbe EO, John EKO, Duque R; Annam O. Resistance to anti tuberculosis drugs in Lagos. Journal of Tropical. Medicine and Hygiene (London). 1992; 95:186-191.

26. Aliyu Gambo, Samer S, El-Kamary, Alash'le Abimiku, Nicholas Ezati, Iwakun Mosunmola, Laura Hungerford, Clayton Brown, Kathleen J. Tracy, Joshua Obasanya and William Blattner: Mycobacterial etiology of pulmonary tuberculosis and association with hiv infection and multidrug resistance in northern Nigeria. Tuberculosis Research and Treatment. 2013; 9. Article ID 650561,

27. Mohammed, Javad Nasiri, Hossein Dabiri, Davood Darban-Sarokhalil, Abdolrazagh Hashemi Shahraki: Prevalence of Non-Tuberculosis Mycobacterial Infections among Tuberculosis Suspects in Iran: Systematic Review and Meta-Analysis. Plos One; 2015. Published:June8, Available:https://doi.org/10.1371/journal.pone.0129073

28. Kendall BA, Winthrop KL: Update on the epidemiology of pulmonary nontuberculous mycobacterial infections. Seminar on Respiratory Critical Care Medicine. 2013; 34:87-94.

29. Thoen C, Lobue P, de Kantor I: The importance of *M. bovis* as a zoonosis. Veterinary Microbiolology. 2006;112(2-4):339-345.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/48876