Original Research Article

PREVALENCE AND RISK FACTORS FOR PULMONARY MYCOBACTERIOSIS IN LAGOS, NIGERIA

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 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 mycobacteriosis 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.

Key words: Pulmonary mycobacteriosis, Mycobacteria, Risk factors, DOTs Centres, Lagos

Abbreviation: DOTs=Directly Observed Therapy Short Course

Background: Mycobacteriosis *is* defined as infection caused by different species of *Mycobacteria* including Non-*Tuberculosis Mycobacteria (NTM) and Mycobacterium Tuberculosis Complex* [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]. 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].

Comment [RRPM1]: The mycobacteriosis is caused by different species of NTM, this way, the mycobacterial of the MTBC are responsible to promote tuberculousis the fact of this bacteria be classified as a mycobacteria do not turn this is as a cause of mycobacteriosis.

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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]. They enhance the non-immunity effect of previous TB exposure [3, 11]. This is also among the challenges faced by the global TB elimination efforts [3].

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 outnumbered *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. There is no doubt that Nigeria require 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.

Methods

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

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.

Ethical considerations: Samples were collected from only participants who voluntarily gave informed consent and were able to submit 2 consecutive sputum samples. The study was also approved by Institutional review Board of the Nigerian Institute of Medical Research, Yaba, Lagos.

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 patient 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 to diabetes was also obtained. Each patient was then screened for HIV 1/II according to the national algorithm [6]. 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 [14], MGIT manual, biochemical tests, immune-chromatographic (ICT) test and line probe molecular assay method.

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

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 [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 as well as 0.5mL into Mycobacteria Growth Indicator Tube 960 [16] containing oleic acid-albumin-dextrose-catalase and polymyxin-amphotericin B-nalidixic acid and trimethoprim-azlocillin. All inoculated media were incubated at 37^0C. 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 media 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).

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 [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].

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

Comment [RRPM3]: here it is ok the use of male and female.

306 (90.3%) were MTBC (Figure 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* (figure 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 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 (Figure 3).

DISCUSSION

More males (59.5%) than female (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

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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 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 15-54years. 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, 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 [28] 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 [30] 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* is inappropriately managed with first-line antituberculous 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 [29]. 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].

Conclusions and recommendations

More males than female had mycobacteriosis in this study. It was also established from this study that mycobacteriosis can be caused by *Mycobacterium tuberculosis complex (MTBC)* and or species of *Non Tuberculosis Mycobacteria* (*NTM*).

Four species of *MTBC* were detected in this study. *M. tuberculosis was the most prevalent species (*80.3%) in the study area followed by *M. bovis* (7.5%), *M. africanum* (9.2%); *and M ulcerans* (2.6%). Also 9.7% of the mycobacteria were *Non-Tuberculosis Mycobacteria (NTM)* consisting of *M. scrofulacium, M.kansasii, M.megateriense, M abscessus, M. fortuitum and M. avium complex* (*M. avium, M. intracellulare, M. colombiense, 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). This study revealed 9.7% of *NTM* mycobacteriosis associated with dry season, HIV and diabetes. The usual TB case detection by microscopy only for DOTs programme could be misleading as exaggerated data on tuberculosis using microscopy alone will be over-diagnosing pulmonary infections caused by *MTBC*, possible false impression of MDRTB and possible inappropriate anti-TB treatment regimen. All sputum smear positive suspected cases of pulmonary mycobacteriosis should be referred for culture, identification and drug susceptibility testing. Capacities for this 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

Comment [RRPM5]: This is a result. I think that it will be better if you change this sentence to item Results.

Comment [RRPM6]: •. I suggest to remove this sentence.
See the correct sentence that you have wrote in "What this study aads?"
" 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 these require special drugs different from the usual first-line anti TB regimen"

Comment [RRPM7]: All this part is a result.

Comment [RRPM8]: This is a conclusion!

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 these require special 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 cases are MDRTB. It may be a case of re-infection with NTMs

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Competing interests: There was no competing interests by the authors in this study.

Authors' contributions: TY Raheem: Designed the proposal, procured the materials and the reagent used for the study, involved in collection of the samples, processing of the samples, data entry and analysis, wrote the manuscript and submitted it for publication.

Iwalokun BA: Supervised the study, involved in the molecular analysis, did data analysis and reviewed the manuscript.

Oluwadun A: Co-supervised the study and reviewed the manuscript.

Adesesan O A: Involved in the sputum culture procedures, identification of the isolated mycobacteria and reviewed the manuscript

Tochukwu N: Involved in the sputum culture procedures and identification of the isolated mycobacteria.

Nshiogu M: Involved in the preparation of the reagent used for the analysis and in the phenotypic identification of the isolated mycobacteria.

Comment [RRPM9]: Not all sputum smear cases are MDRTB.
Of course not. I think that your intention was saying that "not all sputum smear case are caused by mycobacterial from the MTB complex.

Comment [RRPM10]: I suggest that you change this by:
It has to be investigated if the positive sputum smear case is a case of infection with NTMs.

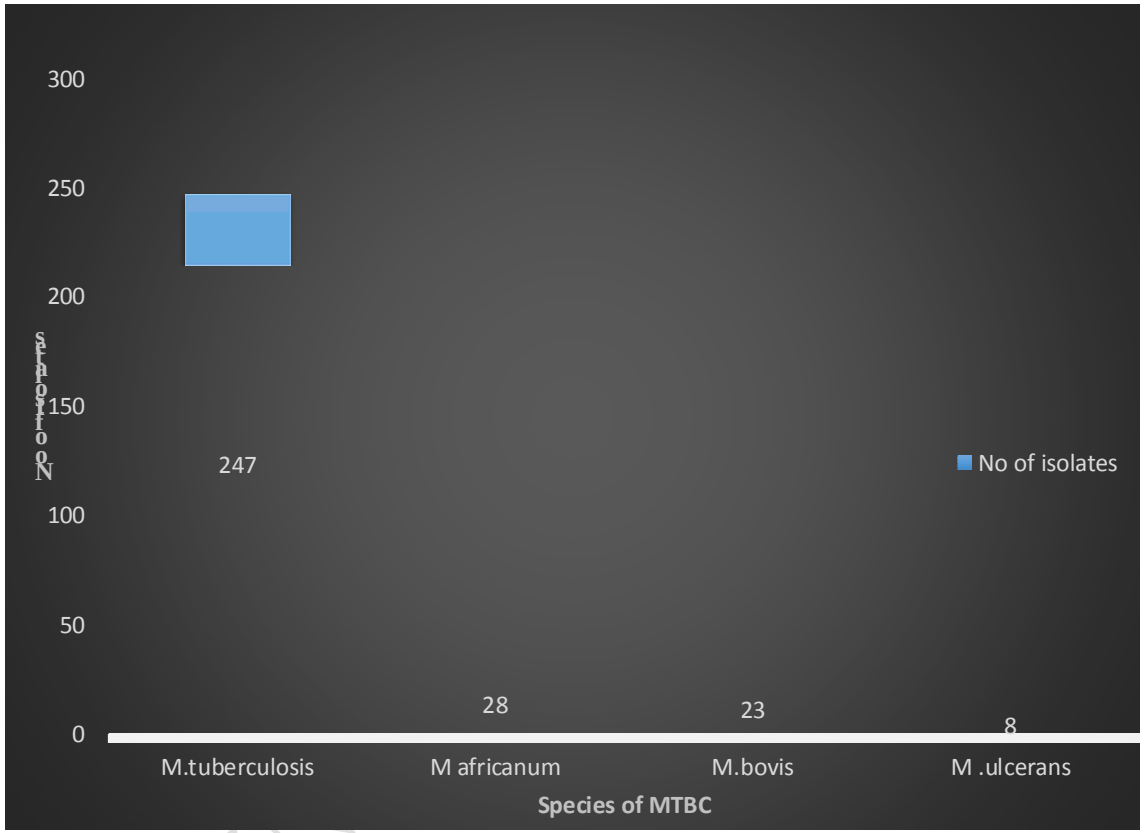

Fig 1: Distribution of the species of MTBC isolated from the participants [p< 0.001]

Comment [RRPM11]: Figure
Remove the color black from the graph.

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

Comment [RRPM12]: I did some changes in shape of Table 3, if you like, use it.

Characteristics	Total isolates N = 339 N (%)	MTB-C N = 306 n (%)	NTM N =33 n (%)	Non-Mycobacterial infection (NMY) N = 43 (%)	P-value (□2 or t-test)
Age group, yr, n (%)					
18 – 35	154(45.4)	140(45.7)	11	34 (79.1)	25.9; <
≥36	185(54.3)	166(54.3)	(33.3)	9 (20.9)	0.0001
			22		
Mean age, yr (mean ± SEM)	34.3+1.5	36.1+1.2	(66.7)	33.4+ 1.4	
			32.5 +		
Gender, n (%)			0.4		
Male	205(60.5)	188(61.4)		25 (58.1)	
Female	134(39.5)	118(38.6)		18 (41.9)	
			20		0.41; 0.81
Education, n (%)			(60.6)		
Primary	122(35.9)	106(34.6)	13(39.4)	28 (65.1)	
Secondary	167(49.3)	165(53.9)		9 (20.9)	
Tertiary	50(14.8)	35(11.4)		6 (14)	27.8; <0.0001
			10		
Occupation, n (%)			(30.3)		
Trading	92(27.1)	87(28.4)	15	11 (25.6)	
Artisan	103(30.3)	86(28.1)	(45.5)	7 (16.3)	
Civil servants	39(11.5)	38(12.4)	8(24.2)	9 (20.9)	28.7;
Private sector	31 (9.1)	28 (9.2)		10 (23.3)	0.00041
worker	74(21.8)	67(21.9)		6 (14)	
Unemployed			9 (27.3)		
			13		

			(39.4)		
Diabetic, n(%)	45(13.3)	76(24.8)	3(9.1)	8 (18.6)	
Yes	294(86.7)	230(75.2)	2 (6.1)	35 (81.4)	
No			6 (18.2)		18.7; < 0.0001
HIV seropositive, n (%)	37 (10.9)	254(83.0)		0 (0)	
Yes	302(89.1)	52 (17.0)	1 (3.0)	43 (100)	
No			32(97.0)		7.6; 0.02
Alcohol intake (%)	56 (16.5)	265(86.6)		5 (11.6)	
	283(83.5)	41(13.4)		38 (88.4)	
Yes			3 (9.1)		
No			30 (90.9)		12.1; 0.002
	61 (18)	50 (16.3)		16 (37.2)	
Smoking, n (%)	278 (82)	256(83.7)		27 (62.8)	
Yes			7(21.2)		
No			26 (78.8)		16.4; 0.0003
Treatment history, n(%)	297(87.6)	64(20.9)		43 (100)	
	42(12.4)	242(79.1)		0 (0)	
Newly diagnosed					
Previously treated			3 (9.1)		10; 0.007
			30 (90.9)		
			27 (81.8)		
			6 (18.2)		

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

Comment [RRPM13]: I did some changes in shape of Table 3, if you like, use it.

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 Diabete.

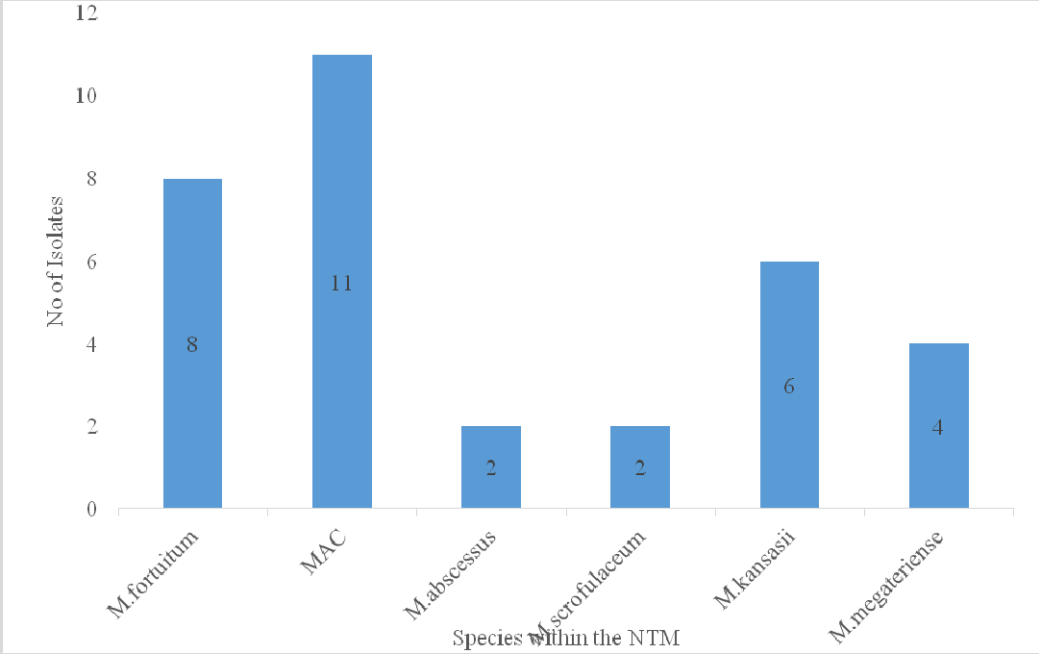

Fig 2: Distribution of Species of NTM isolated from the participants

Comment [RRPM14]: Figure 2

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

Comment [RRPM15]: I did some changes in the format of this Table. If you like, use it.

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)

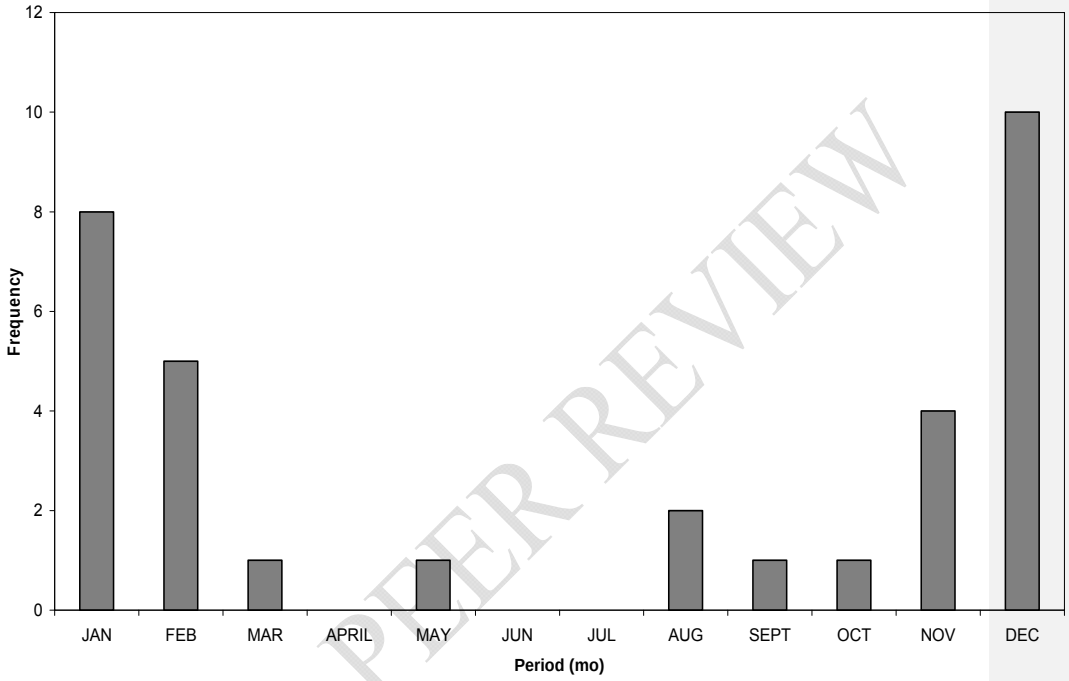

Figure 3: Monthly occurrence of *NTM* infection among the patients with suspected tuberculosis

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