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Prevalence of Nontuberculous Mycobacteria Infections in Patients Diagnosed with Pulmonary Tuberculosis in Ibadan

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Authors' contributions

This study was carried out in collaboration between all authors. Authors OIF and SIBC designed the study. Author OIF managed literature search and data acquisition/analysis and wrote the first draft. Authors OEF and SIBC supervised the work and read the draft. Authors OIF and OEF read and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Nontuberculous mycobacteria (NTM) are mycobacteria *species* other than the *Mycobacterium tuberculosis* complex (MTC); they are opportunistic pathogens and cause life threatening infections with symptoms that mimic those of tuberculosis (TB). Paradoxically, the routine diagnostic tools for TB in Nigeria cannot differentiate MTC from NTM; Hence, TB patients with NTM infections are sometimes incorrectly diagnosed as TB.
Aim: To determine the prevalence of NTM among routinely diagnosed pulmonary TB patients attending the Directly Observed Treatment Short-course (DOTS) centres in Ibadan, Nigeria.
Materials and Methods: This was a cross sectional study conducted at the Tuberculosis Research Laboratory, Department of Veterinary Public Health and Preventive Medicine, University of Ibadan. Sputum samples were collected consecutively from 319 suspected TB patients with or without

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human immunodeficiency virus (HIV) between January 2010 and November 2011 on clinical visits to eight DOTS centres in Ibadan. The samples were cultured on Lowenstein-Jensen medium for about 4-8 weeks after decontamination using N-acetyl-L-cystein-NaOH method. Molecular characterisation of the isolates was done using genus typing. Data obtained were analysed using STATA 12.

Results: In all, a total of 149 isolates were obtained, 26 (17.4%) were identified as NTM; 11 (7.4%) of which were from HIV positive patients, 3 (2.0%) from non HIV patients and 12 (8.0%) unknown cases. The univariable analysis showed association between isolation of NTM and center as well as case. Subjects screened at Ade Oyo (OR=3.5; 95%CI: 1.1-11.8) showed higher likelihood of being infected with NTM.

Conclusion: The high prevalence of NTM among patients earlier diagnosed as PTP following the diagnostic algorithm used in DOTS centers in Nigeria underscores the need for nationwide studies to determine the prevalence of NTM among suspected TB patients. The integration of culture and molecular techniques to improve on the diagnostic algorithm for TB management and control in Nigeria is hereby recommended.

Keywords: Non-tuberculous mycobacteria; tuberculosis; diagnosis; genus typing; patient care.

1. INTRODUCTION

Non-tuberculous mycobacteria (NTM) are environmental or atypical mycobacteria that differ from *Mycobacterium tuberculosis* complex (MTC), the causative agent of tuberculosis (TB) in human and animals and the *Mycobacterium leprae* that is responsible for leprosy disease. These strains of mycobacteria (NTM) are a diverse group of pathogens that is responsible for a substantive but often unappreciated worldwide burden of illness [1]. Although, out of the more than115 characterised NTM species, only a few have been found to be responsible for lung diseases [2]. Pulmonary diseases as a result of NTM most often cause sickness and death [3]. These pathogens are responsible for diseases such as TB-like lung disease; localized infections of the lymphatic system, skin, soft tissue, bone and systemic disease [1]. NTM are ubiquitous and are commonly found in environment such as soil, water sources including municipal water supplies [4,5]. They are presently recognised as important pathogens of humans and are increasingly responsible for human pulmonary infections [5,6]. In 2008, the prevalence of NTM infection in Shanghai was reported to have increased from 4.3% in 2005 to 6.4% in 2008 [7] and in the United States; it has been reported to be important cause of morbidity [5]. Human exposure to NTM is unavoidable due its ubiquitous nature and isolation of NTM is increasingly reported in immunocompetent and immunocompromised hosts [8].

Pulmonary NTM disease shares clinical signs with TB; therefore, causing clinical dilemma with regard to therapy for patients [6]. NTM infections can result in symptomatic disease or asymptomatic colonization, and this distinction can be made by applying the criteria of the American Thoracic Society [9]. Since therapeutic options for NTM differ markedly from those for TB, accurate discrimination of TB from NTM infection is essential to avoid under or over treatment of both conditions bearing in mind the potential patient care and economic consequences. Evidence has revealed that NTMs are responsible for substantial and growing burden of illnesses which are often wrongly diagnosed, thereby affecting the global fight against TB [1].

Nontuberculous mycobacteria are present worldwide; however, most literature on pulmonary nontuberculous mycobacteria (PNTM) disease are from industrialised countries [10] and studies on the burden of illness in industrialized settings have consistently uncovered an unexpectedly high prevalence [1]. However, in many developing countries like Nigeria with high burden and prevalence of TB, the role of NTM in pulmonary disease together with the prevalence of PNTM is largely unknown. The reason for this is because of lack of routine culture and species identification from the samples collected from suspected patients [11,12]. In Nigeria, TB is routinely diagnosed solely on the basis of identification of acid-fast bacilli (AFB) from sputum samples through microscopy; a method that is fraught with low specificity [13]. However, an unknown proportion of such patients could be infected with NTM. Since NTM are resistant to some of the first-line anti-TB medications (Category I drugs), treatment failures occur, leading to treatment with a Category II regimen which involves the use of second line TB drugs [14]. On failure of the latter regimen, such NTM

infected patients may be erroneously diagnosed as multidrug resistant TB (MDR-TB) [15,16]. Currently, there is dearth of information on the prevalence of NTM and their implications in the clinical diagnosis of TB patients in Nigeria. The aim of the study was to determine the prevalence of NTM among the routinely diagnosed pulmonary TB patients attending DOTS centres in Ibadan, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out among suspected/earlier diagnosed TB patients from eight DOTS centres in Ibadan, Oyo State south-western Nigeria. DOTS is the basic package that underpins the Stop TB Strategy and involves the following: secured political commitment with adequate and sustained financing, ensuring early detection and diagnosis through quality-assured bacteriology, provision of standardized treatment with supervision and patient support, ensuring effective drug supply and management, monitoring and evaluating performance impact.

2.2 Mode of Patients Recruitment

Patients recruitment was done through the DOTS centres with the help of health officers. Patients diagnosed to have TB based on Ziehl Neelsen (ZN) and/or X-Ray were recruited for this study.

2.3 Consent and Ethical Approval

Consent was sought from participants who voluntered to participate in the study while ethical approval were obtained from the Oyo State Research Ethical Review Committee, Ministry of Health, Oyo State (NO: AD13/479/71) and the University of Ibadan/University College Hospital Ethical committee (NO: NHREC/05/01/2008a).

2.4 Sample Collection

Sputum samples were collected between January 2010 and November 2011 consecutively from patients on clinical visits to the DOTS centres with the supervision of medical officers at the different centres. Patients were provided with sterile plastic universal bottles into which they voided sputum samples. The bottles were properly labelled to avoid miss-match and transported to the laboratory in ice pack prior to processing. In addition, demographic information on the patients were collected including age, sex and HIV status.

2.5 Laboratory Processing and Mycobacteria Isolation

Processing and culture of sputum samples were done as earlier described by Cadmus et al. [13]. This entailed digestion and decontamination of samples with NALC-(N-acetyl-L-cysteine)-NaOH. Thereafter, processed samples were cultured on Lowenstein Jensen (LJ) media with glycerol or pyruvate and incubated at 37°C for between 4 and 8 weeks or till there was growth.

2.6 Molecular Identification of the Isolates by Genus Typing

This molecular technique for the identification of mycobacteria species is a multiplex PCR method that amplifies species-specific DNA fragments. The technique differentiates MTC from the NTM. It uses six different primers that target a sequence region within the 16S rRNA gene specific for the genus *Mycobacterium*. Genus typing was carried out according to the methods earlier described by Wilton and Cousins [16] and SOP CBU0247 [17]. DNA of the heat killed isolates were amplified and the electrophoresis of the amplified DNA were run on an agarose gel at 100v for one hour**.** The primer design, the PCR amplification and the interpretation of the genus typing is as previously described [18]. All members of genus *Mycobacterium* gives a PCR product at 1030bp while all members of the *Mycobacterium tuberculosis* complex give a PCR product at 372bp and 1030bp.

3. RESULTS

A total of 149 isolates were obtained from the sputum samples cultured on LJ media; out of which 26 (17.4%) were identified as NTM, 122 (81.9%) as MTC and 1 (0.7%) unidentified. Among the patients from whom NTM were isolated, 12 (46.2%) were male patients. Among the patients samples from which NTM were recovered, 11 (42.3%) were co-infected with HIV, three (11.5%) were HIV-negative, while the HIV status of 12 (46.2%) were unknown (Table 1). Among the age group 35-54, 15 (57.7%) NTM was isolated while it was 38.5% among the age group 15-34 years. Summarily, 96.2% of the patients from whom NTM was isolated fell within the age range 15-54 with 84.6% within the age range 20-49 years (Table 2).

The result also showed that 46.2%, 19.2% and 34.6% were isolated from HIV positive patients, HIV negative patients and the patients whose HIV status were unknown respectively whereas, 57.7% and 42.3% of the samples were ZN positive and negative respectively (Table 2). The univariable analysis of the NTM showed a significant association between isolation of NTM and centers with subjects sampled in Ade Oyo (OR=3.5; 95%CI: 1.1- 11.8) being likely to be infected with NTM when compared with those from Jericho Chest Hospital (JCH). Also, we observed a significant association between isolation of NTM and cases, and subjects with new cases being likely to be infected with NTM when compared to those whose cases were not available. Finally, subjects which are HIV positive (OR=3.0; 95%CI:1.2-7.3) are more likely to be infected with NTM when compared to those who are HIV negative (Table 3).

4. DISCUSSION

The isolation of 17.4% NTM from patients diagnosed with TB is note-worthy and underscores the significance of these organisms in the management and control of TB in Nigeria as well as other developing countries where TB diagnosis is still based on smear microscopy. This becomes worrisome given the inherent resistance of NTMs to antitubercular drugs, whereby patients erroneously diagnosed for TB continue to show positive AFB and are hence subjected to other lines of treatment, ultimately resulting in drug resistance.

The prevalence of NTM (17.4%) among suspected/earlier confirmed TB patients in this study is rather high and portends grave public health implications. This findings are particularly important because some of these patients had earlier been diagnosed as TB patients; therefore giving credence to the likelihood of over-diagnosis of TB in this local settings; a trend which may be similar to other settings in Nigeria. This finding may also be similar to situations in other low resource countries with high prevalence of TB where routine diagnosis of TB is solely based on smear microscopy for identification of AFB in sputum samples prior to the initiation of TB treatment. It is therefore plausible to suggest that unknown proportion of patients with NTM infections presented at DOTS centres in Nigeria are routinely wrongly diagnosed and over-diagnosed as TB. This situation is however different from

Table 1. Patients HIV status and the corresponding mycobaterial isolates

HIV status	MTC	NTM	Unidentified
Positive	25 (16.8%)	11 (7.4%)	-
Negative	69 (46.3%)	3 (2.0%)	-
Unclassified	28 (18.8%)	12 (8.0%)	1 (0.7%)
Total	122 (81.9%)	26 (17.4%)	1 (0.7%)

Table 2. Baseline characteristics of case-patients for which NTM was isolated

Characteristics	New, n=9	Retreatment, n=1	Unclassified, n=16	Total, n=26
Age, y				
15-34	5 (55.6%)	0	5 (31.3%)	10 (38.5%)
35-54	4 (44.4%)	1 (100%)	10 (62.5%)	15 (57.7%)
≥ 55	0	0	1 (6.3%)	1 (3.8%)
Sex				
Male	3(33.3%)	0	9 (56.3%)	12 (46.2%)
Female	6 (66.7%)	1 (100%)	7 (43.8%)	14 53.8%)
HIV status				
HIV Positive	2 (22.2%)	1 (100%)	9 (56.3%)	12 (46.2%)
HIV Negative	5 (55.5%)	0	0	5 (19.2%)
Unknown	2 (22.2%)	0	7 (43.8%)	9 (34.6%)
X-Ray result				
Positive	5	1	7	13 (50%)
NA	4	0	9	13 (50%)
ZN Results				
Positive	8	1	6	15 (57.7%)
Negative	1		10	11(42.3%)

Table 3. Univariable analysis of non tuberculous mycobacteria (NTM) in association with centres, age, case and HIV status of subjects

Variable	NTM		OR		P-value
	Positive n(%)	Negative n(%)			
Centres					
Adifase	0 (0.0)	15 (100.0)	0		
JCH	6 (16.7)	30 (83.3)	1		
UCH	4 (40.0)	6 (60.0)	3.3	0.7 – 15.5	0.25
Alafara	3 (13.0)	20 (87.0)	0.8	0.2 – 3.4	0.99
Molet	0 (0.0)	14 (100.0)	0		
Ido	1 (12.5)	7 (87.5)	0.7	0.1 – 6.9	0.81
Olomi	0 (0.0)	2 (100.0)	0		
Adeoyo	10 (41.7)	14 (58.3)	3.5	1.1 – 11.8	0.05*
Egbeda	1 (16.7)	5 (83.3)	1	0.1 – 10.1	0.55
Atolu	2 (22.2)	7 (77.8)	1.4	0.2 – 8.6	0.92
Iwo road	0 (0.0)	2 (100.0)	0		
UI	0 (0.0)	2 (100.0)	0		
Age					
< 19	1 (14.3)	6 (85.7)	1		
20-39	17 (18.7)	74 (81.3)	1.4	0.2 – 12.2	0.82
40-59	8 (21.1)	30 (78.9)	1.6	0.2 – 15.3	0.92
≥ 60	1 (7.7)	12 (92.3)	0.5	0.0 – 9.4	0.75
Sex					
Male	16 (20.8)	61 (79.2)	1		
Female	11 (15.3)	61 (84.7)	0.7	0.3 – 1.6	0.51
Case					
FU	0 (100.0)	3 (100.0)	0		
NA	17 (30.9)	38 (69.1)	1		
New	9 (11.7)	68 (88.3)	0.3	0.1 – 0.7	0.01*
Relapse	1 (7.1)	13 (92.9)	0.2	0.0 – 1.4	0.14
HIV status					
Positive	12 (32.4)	25 (67.6)	3.0	1.2 – 7.3	0.02*
Negative	15 (13.4)	97 (86.6)	1		

**statistically significant at p≤0.05*

that of United States where almost 80% of TB cases are diagnosed on the basis of positive culture results [18,19]. As previously reported, the likelihood is that the current burden of NTM among the TB suspected patients is underestimated in Nigeria due to lack of capacity confirmation using culture, which may also be the case in other developing countries with same limitation of diagnosis of mycobacteria as was reported in Brazil [6].

The prevalence of NTM in this study is lower than the recent 39% in Ibadan [20]. The reason for this difference could be attributed to the lower sample size of only 23 patients used compared to the 149 in the present study. The NTM prevalence in this study is however, higher than the 11% previously reported in Lagos, a city in South-western Nigeria [21]. Furthermore, the NTM prevalence in this study is lower compared to the 23.1%-26.6% range reported in Northern Nigeria [22], but is similar and comparable to the 16.5% recently reported in the south-south region of the country [23] and 15% reported in a similar study [24]. Our findings are also higher than the 11% recently reported in Burkina Faso (a country in West Africa) and Ontario, Canada [12,25] as well as the 6.4% reported from China [7], another vastly developing country though with an improved health care system compared to Nigeria. However, the prevalence of NTM in this study is much lower compared to the 56.9% reported in a study carried out in Taiwan [26].

The public health implication of the findings from this study is that these organisms are resistant to many antimicrobial drugs, even standard TB therapy has been reported not to be effective against NTM infections and this can also complicate treatment of infections [1,27]. In addition, patients that are confirmed to be AFB positive are ordinarily placed on anti-TB therapy.

However, failure of patients to sputum convert from positive to negative after required period under the DOTS programme are treated as multi-drug resistant TB (MDR-TB) patients; and usually treated on very expensive, more toxic and painful second line TB drugs. Several similar cases abound [28,29,30] and of interest are increasing reports in Brazil where some patients who had earlier been wrongly treated as MDR-TB cases were later found to be infected with pulmonary NTM; a trend which is on the increase in Brazil [3]. Furthermore, some industrialised countries have also reported increase prevalence of NTM among patients with pulmonary infections that would have ordinarily been clinically diagnosed as TB [31,32].

In addition, NTMs that were isolated from 84.6% patients within the age range 20-49 years is in agreement with the W.H.O. report that up to two third of TB patients falls within the age range 15-59 years and that the most affected with the disease is the working population [33]. However, this is not in line with the report from Taiwan in which most cases of NTM colonisation was between 65-84 years of age [26]. The result of this study showed that NTM were recovered from the samples of more female than male is similar to the observation in Burkina Faso and Taiwan [14,26].

Our findings further revealed that 11 (42.3%) of the NTM, were isolated from patients co-infected with HIV when compared with only 3 (11.5%) without HIV co-infection. This observation is contrary to the report of a recent study where all the patients from whom NTM were isolated were all HIV-negative [12]. However, previous report showed that these strains of mycobacteria are the commonest opportunistic infection of the immunosuppressed individuals [10]. It has also been noted that climatic and ecological factors as well as the prevalence of co-factors, such as HIV infection, are also likely to influence the prevalence of NTM species [11]. In addition, these strains of mycobacteria are ubiquitous, common in the environment and have been isolated worldwide. The isolation of NTM from the respiratory tract does not per se indicate NTM disease [10]. However NTM are opportunistic pathogens, causing life threatening infections in humans, other mammals and birds [28]. There is therefore, further need to distinguish NTM disease from NTM contamination.

Despite our findings, this present study has some limitations. First, we did not obtain full clinical records of the patients; this could have given us better insight into the clinical presentations of the patients which might have assisted in matching the presentations with the outcome of this study. Second, we were unable to characterize the NTM to species level.

These limitations notwithstanding, our findings revealed a high prevalence of NTM among patients earlier diagnosed for TB in Ibadan, Nigeria. Though Nigeria is a high TB burdened nation, several factors are compromising the ability to evaluate the true burden of the disease. Foremost among this, is the limited capacity and infrastructure required for culture and strain identification of TB prior to treatment.

5. CONCLUSION AND RECOMMENDATION

Therefore, with our findings coupled with earlier ones, it is evident that a significant proportion of suspected TB patients on DOTS programme in the country are infected with NTM. This portends great danger to public health and undermines the health delivery system in Nigeria. Our findings therefore underscore the need to improve on the diagnostic algorithm required for TB diagnosis and more particularly to integrate culture and other modern molecular techniques that can differentiate non-tuberculous mycobacteria from the members of the *Mycobacterium tuberculosis* complex. This will go a long way to improving TB patient care and control in Nigeria. Despite pockets of data from different parts of the country, further research and reports are needed to ascertain the true epidemiological picture of the prevalence of NTM among patients presented at the different DOTS centers in Nigeria. This will go a long way in evaluating the proportion of the over-estimation of TB and MDR-TB among suspected TB patients presenting at DOTS centers in Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Raju RM, Raju SM, Zhao Y, Rubin ER. Leveraging advances in tuberculosis diagnosis and treatment to address nontuberculous mycobacterial disease. Emerging Infectious Diseases. 2016; 22:365-369.
2. Taiwo B, Glassroth J. Nontuberculous mycobacterial lung diseases. Infectious Disease Clinics of North America 2010;24: 769-789.
3. Couto de Mello KG, Mello FCQ, Borga L, Rolla V, Duarte RS, Sampaio EP, Holland SM, Prevots DR, Dalcolmo MP. Clinical and therapeutic features of pulmonary nontuberculous mycobacterial disease, Brazil, 1993–2011. Emerging Infectious Diseases. 2013;19:393-399.
4. Falknham JO III. Epidemiology of infection by nontuberculous mycobacteria. Clinical Microbiology Review. 1996;9:177-215.
5. Mirsaeidi M, Farshidpour M, Allen MB, Ebrahimi G, Falkinham JO. Highlight on advances in nontuberculous mycobacterial disease in North America. BioMedical Research International; 2014. Article ID 919474, 10 pages.
6. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ Jr, Winthrop K. An official ATS/IDSA statement: diagnosis, treatment, and prevention of non tuberculous mycobacterial diseases. American Journal of Respiratory and Critical Care and Medicine. 2007;175:367-416.
7. Wang HX, Yue J, Han M, Yang JH, Gao RL, Jing LJ, Yang SS, Zhao YL, Nontuberculous mycobacteria: susceptibility pattern and prevalence rate in Shanghai from 2005 to 2008. China Medical Journal (English). 2010;123:184-187
8. Glassroth J. Pulmonary disease due to nontuberculous mycobacteria. Chest. 2008;133:243-51.
9. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. American Journal of Respiratory and Critical Care Medicine. 2003;175:367-416.
10. Simons S, Van Ingen J, Hsueh P, Van Hung N, Richard Dekhuijzen PN, Boeree MJ, Van Soolingen D. Nontuberculous mycobacteria in respiratory tract infections, Eastern Asia. Emerging Infectious Diseases. 2011;17:343-3497.
11. Winthrop KL, McNelley E, Kendall B, Marshall-Olson A, Morris C, Cassidy M, Saulson A, Hedberg K. Pulmonary nontuberculous mycobacterial disease prevalence and clinical features: An emerging public health disease. American Journal of Respiratory and Critical Care Medicine. 2010;182:977–982.
12. Borroni E, Badoum G, Cirillo DM, Mattelli A, Moyenga I, Ouedraogo M, Roggi A, Saleri N, Tagliani E, Tortoli E. *Mycobactreium sherrisii* pulmonary disease, Burkina Faso. Emerging Infectious Diseases. 2015;21:2093-2095.
13. Cadmus SIB, Palmer S, Okker M, Dale J, Gover K, Smith N, Jahans K, Hewinson RG, Gordon SV. Molecular analysis of human and bovine tubercle bacilli from a local setting in Nigeria. Journal of Clinical Microbiology. 2006;44(1):29-34.
14. Oswaldo Cruz Foundation/Ministry of Health. Brazil. MDR TB database–Helio Fraga National TB Reference Center; 2013.
    Available:http://sitetb.org/
15. Tabarsi P, Baghaei P, Farnia P, Mansouri N, Chitsaz E, Sheikholeslam F, Marjani M, Rouhani N, Mirsaeidi N, Alipanah N, Amiri M, Masjedi MR, Mansouri D. Nontuberculous mycobacteria among patients who are suspected for multidrug-resistance tuberculosis-need for earlier identification of nontuberculous mycobacteria. American Journal of Medical Science. 2009;337:182-184.
16. Wilton S, Cousins D. Detection and identification of multiple mycobacterial pathogens by DNA amplification in a single tube. *PCR* Methods and Applications. 1992;1:269-273.
17. SOP CBU0247. TB multiplex polymerase chain reaction. VLA-Weybridge, UK; 2005.
18. LoBue PA, Enarson DA, Thoen TC. Tuberculosis in humans and its epidemiology, diagnosis and treatment in the United States. International Journal of Tuberculosis and Lung Diseases. 2010;14: 1226-1232.
19. Winston CA, Mitruka K. Treatment duration for patients with drug-resistant tuberculosis, United States. Emerging Infectious Diseases. 2012;18:1201-1202.

20. Cadmus SI, Diarra B, Traore B, Maiga M, Siddiqui S, Tounkara A, Falodun O, Lawal W, Adewole IF, Murphy R, Soolingen D, Taiwo B. Nontuberculous mycobacteria isolated from tuberculosis suspects in Ibadan, Nigeria. Journal of Pathogens; 2016. Article ID 6547363, 5 pages. Available:http://dx.doi.org/10.1155/2016/6547363
21. Idigbe EO, Anyiwo CE, Onwujekwe DI. Human pulmonary infection with bovine and atypical mycobacteria in Lagos. Journal of Tropical Medicine and Hygiene. 1986;89:143-148.
22. Mawak JD, Gomwalk NE, Bello CSS, Kandakai-Olukemi YT. Human pulmonary infections with bovine and environmental (atypical mycobacteria) in Jos, Nigeria. Ghanian Medical Journal. 2006;40:132-136.
23. Pokam BT, Asuquo AE. 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;1-4.
24. Kim CJ, Kim H, Song NH, Choe PG, Kim ES, Park SW, Kim HB, Kim NJ, Kim EC, Park WB, Oh MD. Differentiating rapid- and slow-growing mycobacteria by difference in time to growth detection in liquid media. Diagnostic Microbiology and Infectious Disease. 2013;75(1):73–76.
25. Machado D, Ramos J, Couto I, Cadir N, Narciso I, Coelho E, Viegas S, Viveiros M. Assessment of the BD MGIT TBc identification test for the detection of *Mycobacterium tuberculosis* complex in a network of mycobacteriology laboratories. BioMedical Research International; 2014. Article ID 398108, 6 pages.
26. Chien J, Lai C, Sheng W, Yu C, Hsueh P. Pulmonary infection and colonization with nontuberculous mycobacteria, Taiwan, 2000-2012. Emerging Infectious Diseases. 2014;20:1382-1385.
27. Idigbe EO, Anyiwo CE, Onwujekwe DI. Human pulmonary infection with bovine and atypical mycobacteria in Lagos. Journal of Tropical Medicine and Hygiene. 1986;89:143-148.
28. Culton DA, Lachiewics AM, Miller BA, Miller MB, MacKuen C, Groben P, White B, Cox GM, Stout JE. Nontuberculous mycobacterila infection after fractionated C02 laser resurfacing. Emerging Infectious Diseases. 2013;19:365-370.
29. Aliyu G, El Kamary SS, Abimiku A, Brown C, Tracy K, Hungerford L, Blattner W. Prevalence of non tuberculous mycobacterial infections among tuberculosis suspects in Nigeria. Zhou D, editor, Plos One. 2013;8e63170. Available:http://dx.doi.org/10.1371/journal,pone.0063170
30. Damaraju D, Jamieson F, Chedore P, Marras TK. Isolation of non-tuberculous mycobacteria among patients with pulmonary tuberculosis in Ontario, Canada. The International Journal of Tuberculosis and Lung Diseases. 2013; 17:676-681
31. Prevots DR, Shaw PA, Strickland D, Jackson LA, Raebel MA, Blosky MA, Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. American Journal of Respiratory and Critical Care Medicine. 2010;182:970-976.
32. Moore JE, Kruijshaar ME, Ormerod LP, Drobniewski F, Aubakar I. Increasing reports of nontuberculous mycobacteria in England, Wales and Northern Ireland. 1995–2006. BMC Pubulic Health. 2010; 10:612.
33. WHO. Global tuberculosis control. WHO/HTM/TB/2011.16 Available:http://whqlibdoc.who.int/publications/2011/978241564380_eng.pdf

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