

Original Research Article

PREVALENCE OF NONTUBERCULOUS MYCOBACTERIA INFECTIONS IN PATIENTS DIAGNOSED WITH PULMONARY TUBERCULOSIS IN IBADAN,

Abstract

Introduction: Nontuberculous mycobacteria (NTM) are *Mycobacterium species* other than the *Mycobacterium tuberculosis* complex (MTC); they are opportunistic pathogens and cause life threatening infections with symptoms that mimics those of tuberculosis (TB). Paradoxically, the routine diagnostic tools for TB in Nigeria cannot differentiate MTC from NTM; Hence, TB could patients with NTM infections are sometimes misdiagnosed as TB.

Aims: 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 human immunodeficiency virus (HIV) between January 2010 and November 2011 on clinical visits to eight DOTS centers 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 was done using genus typing. Data was analysed using STATA 12

Results: In all, 149 isolates were obtained, 26 (17.4%) were identified as NTM; 11 (7.4%) of which were from HIV positive patients, three (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 underscore the need for nationwide studies to determine the prevalence of NTM among suspected TB patients and integration of culture and molecular techniques to improve on the diagnostic algorithm for TB management and control in Nigeria is recommended.

Key words: non-tuberculous mycobacteria, tuberculosis, diagnosis, genus typing, patient care,

34 This work was carried out in collaboration between all authors. Authors OI and SIB designed
35 the study OI managed literature search and data acquisition/analysis and wrote the first draft.
36 OE and SIB supervised the work and read the draft. OI and OE read and approved the final
37 manuscript.

38 **Introduction**

39 Non-tuberculous mycobacteria (NTM) are environmental or atypical mycobacteria that differ
40 from *Mycobacterium tuberculosis* complex (MTC) the causative agent of tuberculosis (TB)
41 in human and animals and the *Mycobacterium leprae* that is responsible for leprosy disease.
42 These strains of mycobacteria (NTM) are a diverse group of pathogens that is responsible for
43 a substantive but often unappreciated worldwide burden of illness [1]. Although, out of the
44 more than 115 characterised NTM species, only a few have been found to be responsible for
45 lung diseases [2]. Pulmonary diseases as a result of NTM most often cause sickness and death
46 [3]. This pathogen is responsible for diseases such as TB-like lung disease; localized
47 infections of the lymphatic system, skin, soft tissue, or bone and systemic disease [1]. NTM
48 are ubiquitous and are commonly found in the environment such as soil, water sources
49 including municipal water supplies [4, 5]. They are presently recognised as important
50 pathogens of humans and are increasingly responsible for human pulmonary infections [5, 6].
51 In 2008, the prevalence of NTM infection was reported to have increased from 4.3% in 2005
52 to 6.4% in 2008 in Shanghai [7] and in the United States; it has been reported to be important
53 cause of morbidity [5]. Human exposure to NTM is unavoidable due its ubiquitous nature and
54 isolation of NTM is increasingly reported in immunocompetent and immunocompromised
55 hosts [8].

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

65 Nontuberculous mycobacteria are present worldwide; however, most literature on PNTM
66 disease are from industrialised countries [10] and studies on the burden of illness in
67 industrialized settings have consistently uncovered an unexpectedly large prevalence [1].
68 However, in many developing countries like Nigeria that has a high burden and prevalence of
69 TB, the role of NTM in pulmonary disease and the prevalence of PNTM is largely unknown.
70 The reason for this is because of lack of routine culture and species identification from the
71 samples collected from suspected patients [11, 12]. In Nigeria, TB is routinely diagnosed
72 solely on the basis of identification of acid-fast bacilli (AFB) from sputum samples through
73 microscopy; a method that is fraught with low specificity [13]. However, an unknown
74 proportion of such patients could be infected with NTM. Since NTM are resistant to some of
75 the first-line anti-TB medications (Category I drugs), treatment failures occur and lead to

76 treatment with a Category II regimen [14]. On failure of the latter regimen, such NTM
77 infected patients may be erroneously diagnosed as multidrug resistant TB (MDR-TB) [15,
78 16]. Currently, there is dearth of information on the prevalence of NTM and their
79 implications in the clinical diagnosis of TB patients in Nigeria. The aim of the study was to
80 determine the prevalence of NTM among the routinely diagnosed pulmonary TB patients
81 attending DOTS centres in Ibadan, Nigeria.

82 **Material and methods**

83 **Study area:** The study was carried out among suspected/earlier diagnosed TB patients from
84 eight DOTS centers in Ibadan, Oyo State south-western Nigeria.

85
86 **Mode of patients recruitment:** Patients recruitment was done through the DOTS centres
87 with the help of health officers. Patients diagnosed to have TB patients based on Ziehl
88 Neelsen (ZN) and/or X-Ray were recruited for this study.

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

94 **Sample collection:** Sputum samples were collected between January 2010 and November
95 2011 consecutively from patients on clinical visits to the DOTS centers with the supervision
96 of medical officers at the different centers. Patients were provided with sterile plastic
97 universal bottles into which they voided sputum samples; was properly labelled to avoid
98 miss-match and transported to the laboratory in ice pack prior to processing. In addition,
99 demographic information on the patients was collected including age, sex and HIV status.

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

105 **Molecular identification of the isolates by Genus typing:**

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

114 *Mycobacterium* gives a PCR product at 1030bp while all members of the *Mycobacterium*
115 *tuberculosis* complex give a PCR product at 372bp and 1030bp.

116 Results

117 A total of 149 isolates were obtained from the sputum samples cultured on LJ media; out of
118 which 26 (17.4%) were identified as NTM, 122 (81.9%) as MTC and 1 (0.7%) unidentified.
119 Among the patients from whom NTM were isolated, 12 (46.2%) were male patients. Among
120 the patients samples from which NTM were recovered, 11 (42.3%) were co-infected with
121 HIV, three (11.5%) were HIV-negative, while the HIV status of 12 (46.2%) were unknown
122 (Table 1). Among the age group 35-54, 15 (57.7%) NTM was isolated while it was 38.5%
123 among the age group 15-34 years. Summarily, 96.2% of the patients from whom NTM was
124 isolated fell within the age range 15-54 with 84.6% within the age range 20-49 years (Table
125 2).

126 The result showed that 46.2% and 19.2% and 34.6% were isolated from HIV positive
127 patients, HIV negative patients and the unclassified cases respectively whereas, 57.7% and
128 42.3% of the samples were ZN positive and negative respectively (Table 2). The univariable
129 analysis of the NTM showed a significant association between isolation of NTM and centers
130 with subjects sampled in Ade Oyo (OR=3.5; 95%CI: 1.1- 11.8) being likely to be infected
131 with NTM when compared with those from JCH. Also, we observed a significant association
132 between isolation of NTM and cases, and subjects with new cases being likely to be infected
133 with NTM when compared to NA. Finally, subjects which are HIV positive (OR=3.0;
134 95%CI:1.2-7.3) are more likely to be infected with NTM when compared to those who are
135 HIV negative (Table 3).

136 **Table 1: Patients HIV status and the corresponding mycobacterial 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%)

142 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%)

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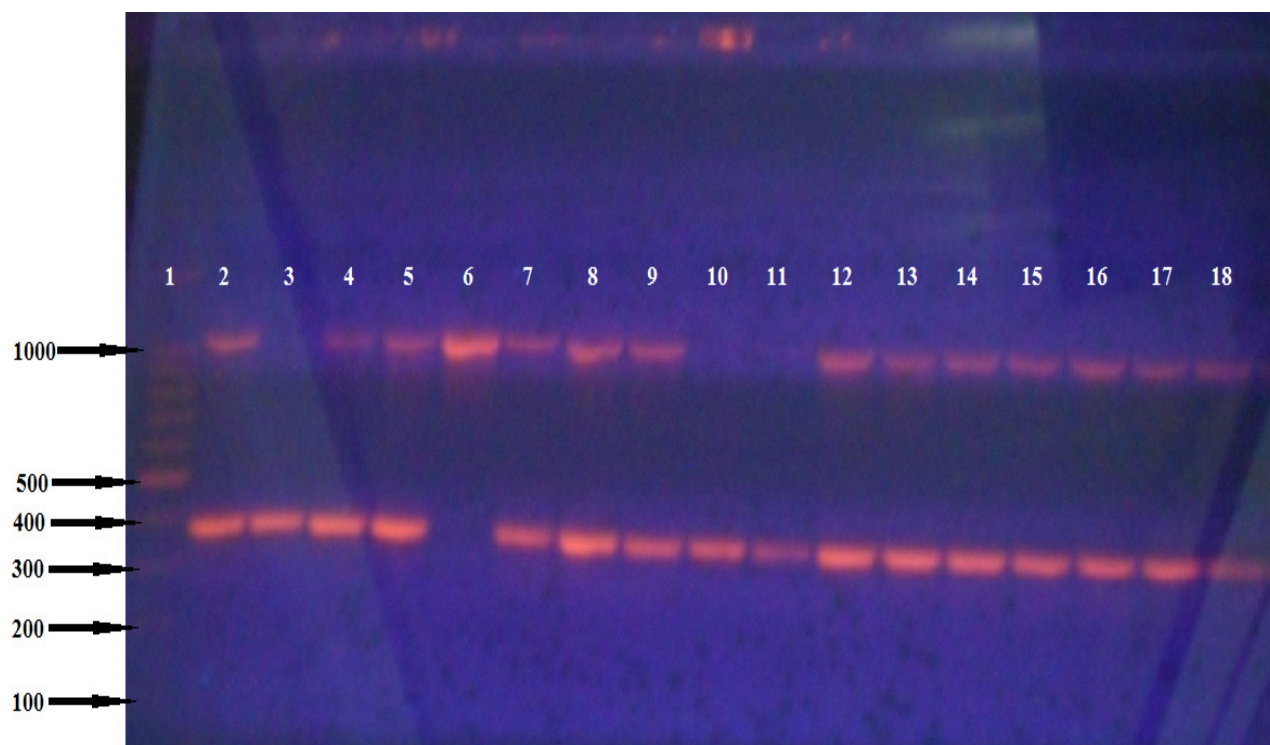
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149 Table 3: Univariable analysis of non tuberculous mycobacteria (NTM) in association with
150 centres, age, case and HIV status of subjects

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		

151 *statistically significant at $p \leq 0.05$



152

153 Plate 4.2. Representative of electrophoretic fractionation of PCR products in 3%
 154 agarose. Lane 1: (DNA Ladder), Lane 2(H37Rv *M. tuberculosis* Reference strain),
 155 Lanes 4-7, 8, 9, 12-18 (*M. tuberculosis* complex; amplification of 372 and 1030bp),
 156 Lanes 3, 10-11 (*M. tuberculosis* complex: amplification of 372bp but 1030 deleted),
 157 Lane 6 (Non tuberculous mycobacteria: Amplification of 1030bp but 372bp deleted)
 158 Lane 6 (Isolate T29/4)

159

160 Discussion

161 The isolation of 17.4% NTM from patients diagnosed with TB is note-worthy and
 162 underscores the significance of these organisms in the management and control of TB in
 163 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 as a whole. 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 centers in Nigeria are routinely mis-diagnosed and over-diagnosed as TB. This situation is however different from 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 there 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 noted in Brazil [6].

The prevalence of NTM in this study is lower than the recent 39% in Ibadan [20]. The reason for this difference may be due 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 previously reported 11% in Lagos [21], another south- western city in Nigeria. Furthermore, the NTM prevalence in this study is lower compared to the 23.1% to 26.6% reported in Northern Nigeria [22], but it is similar and comparable to the 16.5% recently reported in south-south region of the country [23] and the 15% reported from another study in the country [24]. Our findings are also higher than the 11% recently reported respectively in another African country, Burkina Faso and Ontario, Canada [12, 25] as well as the 6.4 % reported from China [7], another vastly developing country though with an improved health system than 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 implications of the findings of this study is that these organisms are resistant to many antimicrobial drugs, even the standard TB therapy have been reported not to be effective against NTM infections which can also complicate treatment of infections [1, 27]. In addition, patients that are confirmed 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 which 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 three (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. Isolating NTM from the respiratory tract does not per se indicate NTM disease [10]. However NTM are opportunistic pathogens and cause life threatening infections in humans, other mammals and birds [34]. 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 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. 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. Finally, 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.

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. *Infect Dis Clin N Am* 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. Mirsaedi M, Farshidpour M, Allen MB, Ebrahimi G, Falkinham JO. Highlight on Advances in Nontuberculous Mycobacterial Disease in North America *BioMedical Research International* Volume 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
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 K.L., McNelley E., Kendall B., Marshall-Olson A., Morris C., Cassidy M., Saulson A. and Hedberg K. Pulmonary nontuberculous mycobacterial disease prevalence and clinical features: an emerging public health disease. *Amer J Respir Crit Care Med* 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, S.I.B., Palmer, S., Okker M., Dale, J., Gover, K., Smith, N., Jahans, K., Hewinson, R.G. and Gordon, S.V. 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 <http://sitetb.org/> 2013
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 Meth Appl* 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 <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.

- 328 26. Chien J, Lai C, Sheng W, Yu C, Hsueh P. Pulmonary infection and colonization with
329 nontuberculous mycobacteria, Taiwan, 2000-2012. *Emerging Infectious Diseases*.
330 2014, 20:1382-1385
- 331 27. Idigbe EO, Anyiwo CE, Onwujekwe DI. Human pulmonary infection with bovine and
332 atypical mycobacteria in Lagos. *Journal of Tropical Medicine and Hygiene*. 1986, 89:
333 143-148
- 334 28. Culton DA, Lachiewicz AM, Miller BA, Miller MB, MacKuen C, Groben P, White B,
335 Cox GM, Stout JE. Nontuberculous mycobacterila infection after fractionated CO2
336 laser resurfacing *Emerging Infectious Diseases*. 2013, 19: 365-370.
- 337 29. Aliyu G, El Kamary S.S., Abimiku A., Brown C., Tracy K., Hungerford L., et al.,
338 2013. Prevalence of non tuberculous mycobacterial infections among tuberculosis
339 suspects in Nigeria. Zhou D, editor, *PLoS ONE* .2013;8e63170.
340 <http://dx.doi.org/10.1371/journal.pone.0063170>
- 341 30. Damaraju D, Jamieson F, Chedore P, Marras TK. Isolation of non-tuberculous
342 mycobacteria among patients with pulmonary tuberculosis in Ontario, Canada *The*
343 *International Journal of Tuberculosis and Lung Diseases*. 2013, 17: 676-681
- 344 31. Prevots DR, Shaw PA, Strickland D, Jackson LA, Raebel MA, Blosky MA,
345 Nontuberculous mycobacterial lung disease prevalence at four integrated health care
346 delivery systems. *Amer J Respir Crit Care Med*. 2010; 182: 970-976.
- 347 32. Moore JE, Kruijsaar ME, Ormerod LP, Drobniewski F, Aubakar I. Increasing
348 reports of nontuberculous mycobacteria in England, Wales and Northern Ireland.
349 1995–2006. *BMC Pubulic Health*. 2010, 10:612.
- 350 33. WHO. Global tuberculosis control. WHO/HTM/TB/2011.16 Available at
351 http://whqlibdoc.who.int/publications/2011/978241564380_eng.pdf
- 352 34. Culton DA, Lachiewicz AM, Miller BA, Miller MB, MacKuen C, Groben P, White B,
353 Cox GM, Stout JE. Nontuberculous mycobacterila infection after fractionated CO2
354 laser resurfacing *Emerging Infectious Diseases* 2013, 19: 365-370.