Isolation and Characterization of Nontuberculous Mycobacteria from Patients with Pulmonary Tuberculosis in Ghana

Isaac Darko Otchere, Adwoa Asante-Poku, Stephen Osei-Wusu, Samuel Yaw Aboagye, Dorothy Yeboah-Manu
Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

Abstract

Objective/Background: Nontuberculous mycobacterial (NTM) species are assuming public health importance in pulmonary diseases; they are increasingly being isolated, and importantly, most NTMs do not respond to routine tuberculosis (TB) drugs. This study aimed to identify NTMs isolated from pulmonary TB cases and also determine their susceptibility to streptomycin (STR), isoniazid (INH), and rifampicin (RIF).

Methods: A total of 1755 mycobacterial isolates, obtained between August 2012 and July 2014, from 2036 smear-positive pulmonary cases were identified using polymerase chain reaction amplification of IS6110, and hsp65 gene sequencing analysis. Drug susceptibility testing (DST) was then performed for the identified NTMs against STR, INH, and RIF using microplate Alamar blue assay. The results were analyzed against patients’ biodata for statistical associations. Results: Of the 1755 analyzed isolates, we identified 43 (2.5%) NTMs, which included 18 (41.9%) Mycobacterium intracellulare, 13 (30.2%) Mycobacterium avium subs. paratuberculosis, 5 (11.3%) Mycobacterium abscessus, 3 (7.0%) each of Mycobacterium mucogenicum and Mycobacterium colombiense, and 1 (2.3%) Mycobacterium simiae. Patients infected with NTMs (52.0%) were more likely to be human immunodeficiency virus-positive (P = 0.001, odds ratio = 6.6, 95% confidence interval = 2.7–16.2) than those infected with *M. tuberculosis* complex (5.8%). All the 43 (100%) NTMs were resistant to INH, whereas 32 (74%) and 19 (44%) were resistant to RIF and STR, respectively. Furthermore, 16 (37.2%) NTMs were resistant to all three drugs, whereas all the *M. avium* isolates were resistant to INH and RIF, but only three were resistant to STR. Among the *M. intracellulare* isolates, 8, 18, and 15 isolates were resistant to STR, INH, and RIF, respectively. Conclusion: The observed high-resistance level to INH and RIF supports the need for rapid species identification and DST of nonresponding TB cases before retreatment.

Keywords: Ghana, *Mycobacterium abscessus*, *Mycobacterium avium*, *Mycobacterium intracellulare*, nontuberculous mycobacteria, pulmonary tuberculosis

Introduction

Nontuberculous mycobacteria (NTMs) species are mycobacteria other than members of the *Mycobacterium tuberculosis* complex (MTBC) and *Mycobacterium leprae*. There are over 170 species identified to date, and unlike *M. tuberculosis*, NTMs are generally free-living, ubiquitous organisms in the environment. The ecology of NTMs makes it easier for human exposure. Until recently, NTMs were not considered clinically important because they were not found to cause diseases. However, lately, the prevalence of clinical NTMs, especially pulmonary NTMs, has been on the increase worldwide. The prevalence rates have been shown to vary among countries globally, and in regions of high tuberculosis (TB) endemicity, NTMs are often unrecognized, misdiagnosed, and/or under diagnosed due to overreliance on smear microscopy. Immune suppression (due to factors, such as human immunodeficiency virus [HIV] infection, and chemotherapy) and underlying lung diseases, such as cystic fibrosis, have been identified as contributing factors for the increasing global incidence of NTMs-associated infections. Although Africa is a TB/HIV-endemic region, information...
Concerning isolation and characterization of pulmonary NTMs, there is a lack of knowledge regarding the contribution of NTMs, which can lead to an overall high rate of misdiagnosis of NTMs as MTBC, since smear microscopy is the most widely used diagnostic tool. NTMs cannot be differentiated from MTBC, and they can also be mistaken for drug-resistant TB. However, there is not a single report on the rate of drug-resistant NTMs.

Most NTMs are intrinsically resistant to the conventional anti-TB drugs, leading to treatment failure of the standard TB regimen. Therefore, NTMs infections are likely to be misdiagnosed as multidrug-resistant TB, and patients are put on second-line anti-TB drugs, which may also fail. Unlike MTBC infections, there is no single treatment regimen for NTMs because of the diversity of spectrum drug resistance influenced by the genetic background of the infecting species/strain. Therefore, it is imperative that proper identification of NTMs is performed to advise an appropriate treatment regimen.

Although smear microscopy is limited by its low sensitivity and discriminatory power, it is the most widely used diagnostic tool for mycobacterial infections, especially in resource-limited countries. Classical identification of NTMs by culture-based biochemical tests has an improved sensitivity and discrimination; however, it is tedious, requires expertise, and the results are sometimes not reproducible. The use of nucleic acid-based typing tools, such as 16S rRNA and hsp65 encoding a 65 kDa protein conserved in all Mycobacteria species, has revolutionized the diagnosis of NTM. The DNA-based assays are rapid, very reproducible, and highly discriminatory for differential diagnosis to advice on species-specific therapy.

We set out to use hsp65 gene sequencing to identify NTMs isolated from pulmonary TB cases in Ghana and to phenotypically test the identified NTMs against isoniazid (INH), rifampicin (RIF), and streptomycin (STR). Our findings suggest that NTMs infections may play a vital role in causing lung diseases, thereby impacting the management of TB in endemic settings such as Ghana.

**Methods**

**Recruitment of study participants**

Sputum smear-positive patients reporting to selected health facilities in the Accra Metropolitan Authority of the Greater-Accra Region and in the northern region (Mampruri East District and Tamale Metropolis) of Ghana between August 2012 and July 2014 were recruited for the study. The procedures used for sample collection were as routinely employed by the National Tuberculosis Program. The structured standard questionnaire was employed to gather patients’ demographic and clinical data. The questionnaire and protocols for this work were reviewed and approved by the Institutional Review Board of the Noguchi Memorial Institute for Medical Research with federal assurance number FWA00001824.

**Cultivation of mycobacteria from sputa**

All specimens were decontaminated using the 5% oxalic acid decontamination method, inoculated on Lowenstein–Jensen medium supplemented with glycerol, incubated at 37°C, and observed for macroscopic growth until 12 weeks. The isolates obtained were suspended in nuclease-free water, deactivated at 98°C for 60 min and spun at 14,000 rpm to pellet cells for DNA extraction.

**Isolation of genomic DNA**

The protocol used for DNA extraction was a hybrid of the protocols as outlined by van Soolingen et al. and Käser et al. Briefly, the mycobacterial cell wall was disrupted by the addition of lysozyme solution (50 µL lysozyme of 10 mg/mL), vortexed, and incubated overnight. Next, 75 µL of 10% SDS and 10 µL of proteinase K (20 mg/mL) was added to the suspension, vortexed, and incubated at 65°C for 15 min. After incubation, 100 µL of 5M NaCl was added to the suspension, followed by CTAB/NaCl which was prewarmed at 65°C, and incubated at 65°C for 10 min. After vortexing, the extracted DNA was purified using the chloroform/isoamyl alcohol extraction method. The DNA contained in the upper phase was precipitated with isopropanol, washed with ethanol, dried, resuspended in 100 µL of Tris-EDTA buffer, and stored at 4°C until use.

**Polymerase chain reaction detection of nontuberculous mycobacteria**

All isolates obtained were typed as MTBC or NTM by polymerase chain reaction (PCR) amplification of the insertion sequence 6110 (IS6110) with MTBC-specific primers as previously described. All IS6110-negative strains were suspected as NTMs and were subsequently used for further analysis.

**Hsp65 gene amplification, DNA sequencing, and blast analysis**

Oligonucleotide primers used for the mycobacterial hsp65 gene were Tb11 (5′-CAACATGGTGTGGTTCCCAT-3′) and Tb12 (5′-CTTGTCAACCGCATACCTTT-3′). Briefly, the PCR reactions contained 3 µL of 10X buffer, 1.8 µL of 15 mM MgCl₂, 3 µL of Q solution, 0.6 µL of 10 mM dNTP mix, 1.8 µL of each primer, 0.2 µL of hot-start Taq polymerase from Qiagen, 14.8 µL of nuclease-free water, and 3 µL of template DNA. Cycling conditions were (a) initial denaturation at 95°C for 15 min and 35 cycles of denaturation at 96°C for 1 min, (b) annealing at 60°C for 1 min, (c) extension at 68°C for 1 min, and (d) final extension at 72°C for 10 min. Amplification of the 441-bp product was confirmed by 1.5% agarose gel electrophoresis and UV transillumination. PCR products were purified and sequenced by outsourcing (Macrogen Europe). Vector sequences in the sequenced hsp65 genes were removed, the sequences were processed into gap experimental files with pregap4, edited with gap4 of the Staden package, and the
corresponding consensus sequence was saved in FASTA format for further analysis. The consensus hsp65 gene sequence of each isolate was analyzed by NCBI nucleotide blast against the microbial database of representative genomes only and selecting highly similar sequences (megablast), whereas leaving all other settings to default.

**Drug susceptibility testing**

Microplate Alamar blue assay (MABA) for STR, INH, and RIF drug susceptibility testing was performed in clear-bottomed, 96-well microplates (Nunc International, Rochester, NY, US). Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. The drug concentrations used were prepared, and the assay was set up by following the standard method,[21,22] with few modifications as described by Otchere et al.[23] and reduced incubation time from 7 to 4 days. All manipulations were conducted in an appropriate, safe working area in the P3 laboratory of the Noguchi Memorial Institute for Medical Research.

**Statistical analysis**

The Fisher’s exact test was used to assess the associations between patient characteristics and species identified. All statistical analyses were performed in STATA 10.1 (Stata Corp., College Station, TX, USA)[24] with significant threshold set to \( P < 0.05 \) at 95% confidence interval (CI).

**RESULTS**

**Patients with nontuberculous mycobacteria infection**

In total, 2036 smear-positive patients were recruited in the study. We excluded 278 from further analysis because no macroscopic cultures were obtained from their sputa after 12 weeks of observation; the remaining 1755 (86.2%) unique isolates were used for further analyses. The characteristics of the study population are summarized in Table 1. Out of 43 patients, comprising 27 (62.8%) male and 16 (37.2%) female patients of average age 39.4 (range, 10–75) years, 43 (2.5%) isolates were identified as NTMs after unsuccessful PCR amplification of IS6110, but positive hsp65 gene amplification [Figure 1]. Furthermore, 25 patients consented to HIV testing, and 13 (52.0%) of them were seropositive. Univariate analysis comparing patients with HIV and NTMs coinfection (52%) and those with HIV and MTBC coinfection (5.8%) showed a statistical association of NTMs infection with HIV \( (P = 0.001, \text{odd ratio [OR]} =15.6, 95\% \text{CI }= 7.7–32.4) \). Among 10 patients who consented to diabetic testing, only 1 (10%) was diabetic. Out of the 25 patients with information on Bacillus Calmette–Guerin, 16 (64%) had the scar.

**Species identity of the isolated nontuberculous mycobacteria**

The DNA sequencing hsp65 gene identified 13 (30.2%) Mycobacterium avium subs. paratuberculosis, 3 (7.0%) each of Mycobacterium colombiense and Mycobacterium mucogenicum, 18 (41.9%) Mycobacterium intracellulare, 5 (11.3%) Mycobacterium abscessus, and 1 (2.3%) Mycobacterium simiae [Figure 2]. No species-specific statistical association was found between the identified NTMs and any of the patient’s clinical data.

**Drug susceptibility profiling of nontuberculous mycobacteria against streptomycin, isoniazid, and rifampicin**

All the 43 NTMs identified were screened for their susceptibility profiles against INH, STR, and RIF using

**Table 1: Biodata of patients from whom nontuberculous mycobacterial species were isolated**

| Feature (number of patients) | \( n \) (\%) |
|------------------------------|--------------|
| Average age (43)             | 39.4 years   |
| Sex (43)                     |              |
| Male                         | 27 (62.8)    |
| Female                       | 16 (37.2)    |
| HIV available (25)           |              |
| Positive                     | 13 (52.0)    |
| Negative                     | 12 (48.0)    |
| HIV not available (18)       |              |
| Diabetes available (10)      |              |
| Positive                     | 1 (10.0)     |
| Negative                     | 9 (90.0)     |
| Diabetes not available (33)  |              |
| BCG scar available (25)      |              |
| Present                      | 16 (64.0)    |
| Absent                       | 9 (36.0)     |
| BCG scar not available (18)  |              |

BCG: Bacillus Calmette–Guerin, HIV: Human immunodeficiency virus

---

Figure 1: Agarose gel of PCR-amplified hsp65 gene products of suspected NTM strains. Lane M is the DNA size marker. Lanes 1–18 are the 441-bp hsp65 gene amplified products from candidate NTM strains. Lane −ve is the negative PCR control. Lane +ve is the positive control amplified from Mycobacterium tuberculosis H37Rv. NTM: Nontuberculous mycobacterial species, PCR: Polymerase chain reaction.
MABA. All 43 (100%) identified NTMs were resistant to INH, 26 (60.5%) were resistant to all the 3 drugs tested, 11 (25.6%) were resistant to both INH and RIF, and 3 (6.9%) were resistant to RIF and STR in addition to INH resistance. Moreover, 3 (6.9%) of the isolates were susceptible to STR and RIF, 6 (13.9%) were susceptible to RIF irrespective of INH and STR resistance, and none was susceptible to INH [Table 2].

Stratifying drug profile by species, all the 13 M. avium subs. paratuberculosis strains were resistant to INH and RIF irrespective of INH resistance, whereas 7 (53.8%) were additionally resistant to STR. All the 5 M. abscessus (100%) strains were resistant to all drugs, whereas 12 (66.7%), 18 (100%), and 16 (88.9%) of the M. intracellulare strains were resistant to STR, INH, and RIF, respectively. All the 3 M. colombiense strains were resistant to INH, whereas 2 (66.7%) and 1 (33.3%) were resistant to STR and RIF, respectively. While all the 3 M. mucogenicum strains were additionally resistant to STR, 2 (66.7%) out of the 3 were additionally resistant to RIF. Last, the single M. simiae strain was susceptible to STR and RIF, but resistant to INH [Table 3].

**Discussion**

Pulmonary infections caused by NTMs are often misdiagnosed as conventional TB caused by members of the MTBC because of the nonspecificity of sputum smear microscopy.\(^{1-6}\) In addition, because of their intrinsic resistance to most first-line anti-TB drugs,\(^{9,10}\) NTMs-associated TB may be normally diagnosed as TB and managed as MDR with its associated public health implications.\(^{25-28}\) In Ghana, the prevalence and epidemiology of NTMs are largely unknown.\(^{25}\) We sought to (1) determine the proportion and identity of NTMs among our prospective collection of mycobacteria isolates from smear-positive pulmonary TB cases reporting to selected health facilities in Ghana; (2) establish their susceptibility to the anti-TB drugs INH, RIF, and STR; and (3) analyze for possible association of NTMs infection with patient characteristics. We found that (1) 2.5% of our mycobacterial isolates were NTMs and were dominated by M. intracellulare; (2) most of the identified NTMs were resistant to the two backbone drugs (INH and RIF) of the Directly Observed Treatment Short-course (DOTS) regimen; and (3) there was a significant association between NTMs-infected patients and HIV. Compared with the global incidence and a recent report on NTMs from Ghana, which identified 8% of NTMs among HIV, compared with the global incidence and a recent report on NTMs from Ghana, which identified 8% of NTMs among clinical mycobacteria from presumptive TB cases, our report of 2.5% is relatively small.\(^{29-32}\) However, the previous study in Ghana involved only HIV-positive presumptive TB patients, which may explain the high rate of NTMs from that study. Nevertheless, for individual patient care and public health purposes, 2.5% is still high enough to reiterate the importance of rapid identification of the infecting species to facilitate effective treatment of pulmonary TB cases.

Our data set was dominated by M. intracellulare and M. avium subs. paratuberculosis [Figure 2], which constitute the M. avium complex (MAC). This finding compares with earlier reports of the dominance of MAC among pulmonary NTM infections.\(^{1,11,29-32}\) This result is suggestive of the possible dominance of the MAC species in the environmental settings of the country. In addition, our results give more credit to the use of hsp65 gene sequence analysis for rapid identification of NTMs [Figure 2]. Compared with the earlier report from Ghana that could identify 17/38 (44.7%) of the NTMs using genotyping tools other than hsp65 gene sequencing,\(^{32}\) we were able to identify all the isolated NTMs using the hsp65 gene

---

**Table 2: Distribution of drug resistance among nontuberculous mycobacterial species**

| Resistance                  | n (%)        |
|-----------------------------|--------------|
| INH + RIF + STR             | 26 (60.5%)   |
| INH + STR                   | 3 (6.9%)     |
| RIF + INH                   | 11 (25.6%)   |
| INH: Isoniazid, RIF: Rifampicin, STR: Streptomycin |

**Table 3: Drug susceptibility profile of identified nontuberculous mycobacterial species**

| Species (n)                        | STR (%) | INH (%) | RIF (%) |
|------------------------------------|---------|---------|---------|
| M. avium paratuberculosis (13)     | 7 (53.8)| 13 (100)| 13 (100)|
| M. colombiense (3)                 | 2 (66.7)| 3 (100) | 1 (33.3)|
| M. intracellulare (18)             | 12 (66.7)| 18 (100)| 16 (88.9)|
| M. abscessus (3)                   | 5 (100) | 5 (100) | 5 (100) |
| M. mucogenicum (3)                 | 3 (100) | 3 (100) | 2 (66.7)|
| M. simiae (1)                      | -       | 1 (100) | -       |
| Total number of isolates           | 29 (67.4)| 43 (100)| 37 (86.0)|
| INH: Isoniazid, RIF: Rifampicin, STR: Streptomycin: M. intracellulare, M. mucogenicum: Mycobacterium avium, M. simiae: Mycobacterium simiae |

---

**Figure 2:** Distribution of identified NTMs. NTM: Nontuberculous mycobacterial species.
sequencing. Our study, therefore, highlights the discriminatory power of the use of hsp65 gene sequencing for mycobacterial speciation.

Individuals that are immunocompromised due to HIV infection are at a risk of NTMs infection as evidenced by our study. We found a significant association between NTMs-infected patients and HIV (P = 0.001, OR = 15.6, 95% CI = 7.7–32.4). This finding supports earlier reports implicating the global HIV pandemic on the increasing global cases of NTMs-associated infections[33,34] and also compares with earlier works done in Tanzania and South Africa,[29,35] where HIV co-infection was implicated as a predictor of pulmonary NTMs infections. It is, therefore, imperative that TB treatment failures on the standard DOTs, especially among HIV-coinfected patients, are properly investigated to exclude the possibility of treating NTMs infection as an MDR-TB case.

NTMs pose a major challenge for TB treatment programs since such patients are managed mainly on the basis of smear microscopy,[9] which cannot determine their drug susceptibility profiles leading to their misdiagnoses as MDR-TB cases. Most of the NTMs (60.5%) identified were resistant to all the three drugs (INH, STR, and RIF), whereas 11 (25.6%) were resistant to the two most important drugs of the DOTS regimen (INH and RIF). None of the 43 isolates was sensitive to INH. This result is in agreement with the already established high resistance of NTMs to the drugs commonly used in the management of pulmonary TB cases[36,37] and reinforces the need to correctly identify the causative agent (to the species level) of such patients before they are put on an appropriate treatment regimen. Comparing independent resistance to the three drugs (43 INH, 29 STR, and 37 RIF) suggests that STR was the most active drug against our identified NTMs [Table 3] even though the proportion of identified NTMs resistant to STR (67.4%) was still high. Among the identified species, M. abscessus was the only species that had all strains resistant to all the three drugs [Table 3] and had the highest minimal inhibitory concentrations (S1). This finding supports earlier reports of resistance of M. abscessus to various antimicrobials, hence, making the treatment of M. abscessus infections very difficult.[9,38,39] This extreme resistance of M. abscessus has been partly attributed to the unique genetic constitution, probably due to horizontal gene transfer, a trait that is largely missing in the MTBC Supplementary Table 1.[39,42]

**CONCLUSION**

Our results emphasize the importance of NTMs in pulmonary infections, which is dominated by the M. intracellulare complex. The high level of resistance of the identified species to INH and RIF calls for rapid identification and susceptibility testing of the causative agents of nonconverting TB patients or treatment failures for proper management.

**Financial support and sponsorship**

This study was fully funded by the Wellcome Trust Intermediate Fellowship 97134/Z/11/Z awarded to DYM.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Nunes-Costa D, Alarico S, Dalcolmo MP, Correia-Neves M, Empadinhas N. The looming tide of nontuberculous mycobacterial infections in Portugal and Brazil. Tuberculosis (Edinb) 2016;96:107-19.
2. Falkingham JO 3rd. Environmental sources of nontuberculous mycobacteria. Clin Chest Med 2015;36:35-41.
3. Shinnick TM, Good RC. Mycobacterial taxonomy. Eur J Clin Microbiol Infect Dis 1994;13:884-901.
4. Desikan P. Sputum smear microscopy in tuberculosis: Is it still relevant? Indian J Med Res 2013;137:442-4.
5. Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, Cunningham J, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: A systematic review. Lancet Infect Dis 2006;6:664-74.
6. Perkins MD, Cunningham J. Facing the crisis: Improving the diagnosis of tuberculosis in the HIV era. J Infect Dis 2007;196 Suppl 1:S15-27.
7. Falkingham JO 3rd. Surrounded by mycobacteria: Nontuberculous mycobacteria in the human environment. J Appl Microbiol 2009;107:356-67.
8. World Health Organization. Global Tuberculosis Report 2014. WHO/HTM/TB/2014.08. Geneva, Switzerland: World Health Organization; 2014.
9. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. American Thoracic Society Documents An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases; 2007.
10. Griffith DE. Nontuberculous mycobacterial lung disease. Curr Opin Infect Dis 2010;23:185-90.
11. Lee MR, Sheng WH, Hung CC, Yu CJ, Lee LN, Hsuw PR. Mycobacterium abscessus complex infections in humans. Emerg Infect Dis 2015;21:1638-46.
12. Griffith DE. Mycobacterium abscessus subsp abscessus lung disease: ‘Trouble ahead, trouble behind…’. F1000Prime Rep 2014;6:107.
13. Yeboah-Manu D, Bodmer T, Mensah-Quainoo E, Owusu S, Ofori-Adjei D, Pluschke G. Evaluation of decontamination methods and growth media for primary isolation of Mycobacterium ulcerans from surgical specimens. J Clin Microbiol 2004;42:5875-6.
14. WuTS, Lu CC, Lai HC. Current situations on identification of nontuberculous mycobacteria. J Biomed Lab Sci 2009;21:1-6.
15. Saifi M, Jabbarzadeh E, Bahrmand AR, Karimi A, Pourazar S, Fateh A, et al. HSP65-PRA identification of non-tuberculosis mycobacteria from 4892 samples suspicious for mycobacterial infections. Clin Microbiol Infect 2013;19:723-8.
16. Galagan JE. Genomic insights into tuberculosis. Nat Rev Genet 2004;15:307-20.
17. van Soolingen D, Hermans PW, de Haas PE, Soll DR, van Embden JD. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: Evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol 1991;29:2578-86.
18. Käsper M, Ruf MT, Hauser J, Marsollier L, Pluschke G. Optimized method for preparation of DNA from pathogenic and environmental mycobacteria. Appl Environ Microbiol 2009;75:414-8.
19. Asante-Poku A, Otchere ID, Danso E, Mensah DD, Bonsu F, Gagneux S, et al. Evaluation of GenoType MTBDRplus for the rapid detection of drug-resistant tuberculosis in Ghana. Int J Tuberc Lung Dis 2015;19:954-9.
20. Staden R, Beal KF, Bonfield JK. The Staden package, 1998. Methods Mol Biol 2000;132:115-30.
21. Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F. Evaluation of decontamination methods and 'Trouble ahead, trouble behind…'. F1000Prime Rep 2014;6:107.
22. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, et al. Rapid, low-technology MIC determination with
Otchere, et al.: Drug resistant non-tuberculous mycobacteria among pulmonary TB cases: A prospective study in Ghana

clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. J Clin Microbiol 1998;36:362-6.

23. Otchere ID, Asante-Poku A, Osei-Wusu S, Baddoo A, Sarpong E, Ganiyu AH, et al. Detection and characterization of drug-resistant conferring genes in *Mycobacterium tuberculosis* complex strains: A prospective study in two distant regions of Ghana. Tuberculosis (Edinb) 2016;99:147-54.

24. Tomz M, Wittenberg J, King G. Clarify: Software for interpreting and presenting statistical results. J Stat 2003;8:1-28.

25. Couto I, Machado D, Viveros M, Rodrigues L, Amaral L. Identification of nontuberculous mycobacteria in clinical samples using molecular methods: A 3-year study. Clin Microbiol Infect 2010;16:1161-4.

26. Adjemian J, Oliveira KN, Prevots DR. Nontuberculous mycobacteria among patients with cystic fibrosis in the United States: Screening practices and environmental risk. Am J Respir Crit Care Med 2014;190:581-6.

27. Fusco da Costa AR, Lopes ML, de Sousa MS, Suffys PN, Sales LHM, Lima KVB. Pulmonary Nontuberculous Mycobacterial Infections in the State of Pará, an Endemic Region for Tuberculosis in North of Brazil, Pulmonary Infection, Dr. Amer Amal (Ed.). ISBN: 978-953-51-0286-1, InTech. 2012. Available from: http://www.intechopen.com/books/pulmonaryinfection/pulmonary-nontuberculous-mycobacterial-infections-in-the-state-of-par-an-endemic-region-for-tuberculosis. [Last accessed on 2016 May 27].

28. da Costa AR, Lopes ML, Leão SC, Schneider MP, de Sousa MS, Suffys PN, et al. Molecular identification of rapidly growing mycobacteria isolates from pulmonary specimens of patients in the State of Pará, Amazon region, Brazil. Diagn Microbiol Infect Dis 2009;65:358-64.

29. Hoza AS, Mfinanga SG, Rodloff AC, Moser I, König B. Increased isolation of nontuberculous mycobacteria among TB suspects in Northeastern, Tanzania: Public health and diagnostic implications for control programmes. BMC Res Notes 2016;9:109.

30. Zamarrioli LA, Coelho AG, Pereira CM, Ferrazoli L, Bammann RH. Laboratory identification of mycobacteria in respiratory samples from HIV-positive patients suspected of tuberculosis. Rev Soc Bras Med Trop 2009;42:290-7.

31. Lima CA, Gomes HM, Oelemann MA, Ramos JP, Caldas PC, Campos CE, et al. Nontuberculous mycobacteria in respiratory samples from patients with pulmonary tuberculosis in the state of Rondônia, Brazil. Mem Inst Oswaldo Cruz 2013;108:457-62.

32. Bjerrum S, Oliver-Commey J, Kenu E, Larrey M, Newman MJ, Addo KK, et al. Tuberculosis and non-tuberculous mycobacteria among HIV-infected individuals in Ghana. Trop Med Int Health 2016;21:1181-90.

33. Ferreira RM, Saad MH, Silva MG, Fonseca Lde S. Non-tuberculous mycobacteria I: One year clinical isolates identification in Tertiary Hospital Aids Reference Center, Rio de Janeiro, Brazil, in pre highly active antiretroviral therapy era. Mem Inst Oswaldo Cruz 2002;97:725-9.

34. Senna SG, Marsico AG, Vieira GB, Sobral LF, Suffys PN, Fonseca Lde S. Identification of nontuberculous mycobacteria isolated from clinical sterile sites in patients at a university hospital in the city of Rio de Janeiro, Brazil. J Bras Pneumol 2011;37:521-6.

35. Sookan L, Coovadia YM. A laboratory-based study to identify and speculate non-tuberculous mycobacteria isolated from specimens submitted to a central tuberculosis laboratory from throughout KwaZulu-Natal Province, South Africa. S Afr Med J 2014;104:766-8.

36. World Health Organization. Global Tuberculosis Report 2013. Geneva, Switzerland: World Health Organization; 2013. p. 306.

37. Viveiros M, Leandro C, Amaral L. Mycobacterial efflux pumps and chemotherapeutic implications. Int J Antimicrob Agents 2003;22:274-8.

38. Cândido PHP, Nunes Lde S, Marques EA, Folescu WS, Coelho FS, de Moura VC, et al. Multidrug-resistant nontuberculous mycobacteria isolated from cystic fibrosis patients. J Clin Microbiol 2014;52:2990-7.

39. Nessar R, Cambau E, Reyrat JM, Murray A, Gicquel B. Mycobacterium abscessus: A new antibiotic nightmare. J Antimicrob Chemother 2012;67:810-8.

40. Nober A, Alarico S, Maranhao A, Mendes V, Empadinas N. The molecular biology of mycobacterial trehalose in the quest for advanced tuberculosis therapies. Microbiology 2014;160( Pt 8):1547-70.

41. Shallom SJ, Gardina PJ, Myers TG, Sebastian Y, Conville P, Calhoun LB, et al. New rapid scheme for distinguishing the subspecies of the *Mycobacterium abscessus* group and identifying *Mycobacterium massiliense* isolates with inducible clarithromycin resistance. Int Microbiol 2013;16:2943-9.

42. Macheras E, Roux AL, Bastian S, Leão SC, Palaci M, Sivadon-Tardy V, et al. Multilocus sequence analysis and rpoB sequencing of *Mycobacterium abscessus* (sensu lato) strains. J Clin Microbiol 2011;49:491-9.
## Supplementary Table

### Supplementary Table 1: Patient demography of NTMs

| PCR number | First hit          | Percentage identity | E | MIC | Sensitivity | Gender | Age | HIV | Occupation | BCG | Diabetes |
|------------|--------------------|---------------------|---|-----|-------------|--------|-----|-----|------------|-----|----------|
| 1          | *M. avium*         | 99.00               | 0.0 | 2   | >1          | R      | R   | R   | Female     | 39  |          |
| 2          | *M. avium*         | 100.00              | 0.0 | 1   | >1          | 2      | S   | R   | R          | Male | 47       |
| 3          | *M. intracellulare*| 99.00               | 0.0 | 1   | 0.5        | 0.06   | S   | R   | S          | Male | 38       |
| 4          | *M. avium*         | 99.00               | 0.0 | 2   | >1          | >2     | R   | R   | R          | Female| 51       |
| 5          | *M. avium*         | 99.00               | 0.0 | >4  | >1          | 2      | R   | R   | R          | Male | 10       |
| 6          | *M. avium*         | 99.00               | 0.0 | 1   | >1          | >2     | S   | R   | R          | Male | 33       |
| 7          | *M. avium*         | 99.00               | 0.0 | 1   | >1          | 2      | S   | R   | R          | Female| 19       |
| 8          | *M. avium*         | 99.00               | 0.0 | 2   | >1          | >2     | R   | R   | R          | Male | 27       |
| 9          | *M. intracellulare*| 99.00               | 0.0 | >4  | >1          | 2      | R   | R   | R          | Female| 32       |
| 10         | *M. avium*         | 99.00               | 0.0 | 4   | >1          | >2     | R   | R   | R          | Male | 42       |
| 11         | *M. simiae*        | 99.00               | 0.0 | 0.5 | >1          | 0.06   | S   | R   | S          | Male | 52       |
| 12         | *M. avium*         | 99.00               | 0.0 | 2   | >1          | >2     | R   | R   | R          | Male | 24       |
| 13         | *M. intracellulare*| 99.00               | 0.0 | >4  | >1          | 1      | R   | R   | R          | Male | 35       |
| 14         | *M. colombiense*   | 99.00               | 0.0 | 1   | >1          | 0.12   | S   | R   | S          | Female| 46       |
| 15         | *M. abscessus*     | 100.00              | 0.0 | >4  | >1          | >2     | R   | R   | R          | Male | 72       |
| 16         | *M. intracellulare*| 99.00               | 0.0 | >4  | >1          | 0.5    | R   | R   | R          | Female| 32       |
| 17         | *M. abscessus*     | 99.00               | 0.0 | 4   | >1          | >2     | R   | R   | R          | Male | 53       |
| 18         | *M. avium*         | 100.00              | 0.0 | >4  | >1          | >2     | R   | R   | R          | Male | NA       |
| 19         | *M. avium*         | 100.00              | 0.0 | 1   | >1          | 2      | S   | R   | R          | Male | 24       |
| 20         | *M. avium*         | 99.00               | 0.0 | 1   | >1          | >2     | S   | R   | R          | Male | 25       |
| 21         | *M. abscessus*     | 99.00               | 0.0 | >4  | >1          | >2     | R   | R   | R          | Female| 46       |
| 22         | *M. abscessus*     | 99.00               | 0.0 | >4  | >1          | >2     | R   | R   | R          | Male | 37       |
| 23         | *M. mucogenicum*   | 99.00               | 0.0 | >4  | >1          | >2     | R   | R   | R          | Female| 33       |
| 24         | *M. intracellulare*| 99.00               | 0.0 | >4  | >1          | >2     | R   | R   | R          | Female| 75       |
| 25         | *M. intracellulare*| 99.00               | 0.0 | 4   | >1          | >2     | R   | R   | R          | Male | 38       |
| 26         | *M. mucogenicum*   | 99.00               | 0.0 | 2   | >1          | 0.5    | R   | R   | R          | Male | 32       |
| 27         | *M. avium*         | 99.00               | 0.0 | 0.5 | >1          | >2     | S   | R   | R          | Female| 29       |
| 28         | *M. colombiense*   | 99.00               | 0.0 | 4   | >1          | 0.5    | R   | R   | R          | Male | 34       |
| 29         | *M. intracellulare*| 99.00               | 0.0 | >4  | >1          | 0.12   | R   | S   | R          | Female| 33       |
| 30         | *M. intracellulare*| 99.00               | 0.0 | 1   | >1          | 2      | S   | R   | R          | Female| 33       |
| 31         | *M. abscessus*     | 100.00              | 0.0 | >4  | >1          | >2     | R   | R   | R          | Female| 46       |
| 32         | *M. colombiense*   | 99.00               | 0.0 | 2   | >1          | 0.06   | R   | R   | S          | Male | 70       |
| 33         | *M. mucogenicum*   | 99.00               | 0.0 | 4   | >1          | 0.06   | R   | R   | S          | Male | 70       |
| 34         | *M. intracellulare*| 100.00              | 0.0 | 2   | >1          | >2     | R   | R   | R          | Male | 28       |
| 35         | *M. intracellulare*| 99.00               | 0.0 | 1   | >1          | 1      | S   | R   | R          | Male | 18       |
| 36         | *M. intracellulare*| 100.00              | 0.0 | 1   | >1          | 1      | S   | R   | R          | Male | 55       |
| 37         | *M. intracellulare*| 99.00               | 0.0 | 2   | >1          | >2     | R   | R   | R          | Female| 31       |
| 38         | *M. intracellulare*| 99.00               | 0.0 | 1   | >1          | 1      | S   | R   | R          | Female| 40       |
| 39         | *M. intracellulare*| 100.00              | 0.0 | >4  | >1          | 0.5    | R   | R   | R          | Male | NA       |
| 40         | *M. intracellulare*| 99.00               | 0.0 | >4  | >1          | >2     | R   | R   | R          | Female| 33       |
| 41         | *M. intracellulare*| 100.00              | 0.0 | 1   | 1          | 1      | S   | R   | R          | Female| 56       |
| 42         | *M. intracellulare*| 99.00               | 0.0 | 2   | >1          | >2     | R   | R   | R          | Male | 32       |
| 43         | *M. intracellulare*| 100.00              | 0.0 | 4   | >1          | >2     | R   | R   | R          | Female| 31       |

BCG: Bacillus Calmette-Guerin, HIV: Human immunodeficiency virus, INH: Isoniazid, RIF: Rifampicin, STR: Streptomycin, MIC: Minimum inhibitory concentration, S: Sensitive, R: Resistant, I: Intermediate, NA: Not available, ND: Not deducted, PCR: Polymerase chain reaction, *M. intracellulare*: Mycobacterium intracellulare, *M. mucogenicum*: Mycobacterium mucogenicum, *M. colombiense*: Mycobacterium colombiense, *M. abscessus*: Mycobacterium abscessus, *M. avium*: Mycobacterium avium