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Nontuberculous Mycobacterial Diseases among People Living with HIV (PLHIV) at the Philippine General Hospital

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ABSTRACT

Background: *Diagnosing nontuberculous mycobacterial (NTM) disease especially among people living with HIV (PLHIV) is a challenge as misdiagnosis or underdiagnosis can happen in TB-endemic countries like the Philippines. To the best of our knowledge, this is the first local study describing the clinical, radiologic and microbiologic profile of NTM infections among patients with HIV/AIDS.*

Methods: *This descriptive study was conducted in the University of the Philippines-Philippine General Hospital (UP-PGH) involving PLHIV with NTM growth in their mycobacterial cultures done from November 1, 2013 to January 31, 2016. Available cultures with NTM growth from the Central Laboratory and Medical Research Laboratory were retrieved and underwent speciation by REBA Myco-ID and drug susceptibility testing (DST). Medical charts of these PLHIV with speciated NTM were reviewed for demographic and clinical data including signs and symptoms, past medical and personal history, HIV treatment status, laboratory results, and imaging findings. The diagnosis of NTM disease among patients were classified into definite NTM disease (pulmonary, extrapulmonary and disseminated), possible NTM disease, and not NTM disease. Treatment and clinical outcomes were also reviewed thereafter.*

Results and Conclusions: *There were 330 specimens from PLHIV submitted for mycobacterial cultures from November 1, 2013 to end of January 2016 - with 315 to the Central Laboratory and 15 to the Medical Research Laboratory. We retrieved 50 cultures with known NTM growth from different specimens: 46 from sputum, 2 from stool, 1 from tissue and 1 from abscess. Using the REBA Myco-ID test kit, we identified the specific NTM species in 39 of the 50 cultures. The rest turned out to be MTB on speciation (6) or had test failures or remained unidentified (5). The most common NTM specie, regardless of clinical significance was Mycobacterium fortuitum (15) followed by M. avium (9) and then M. mucogenicum (5). DST was done in 35 NTM isolates. All isolates of Mycobacterium avium complex (M. avium + M. intracellulare) were susceptible to clarithromycin but resistant to ethambutol and isoniazid while M. fortuitum was 100% sensitive to both ciprofloxacin and amikacin. Medical charts of 33 out of the 39 patients with NTM were reviewed and were categorized into definite NTM disease in 9 cases (8 pulmonary NTM and 1 extrapulmonary NTM), possible NTM disease in 15 cases and not NTM disease in 9 cases. Fever (79%), chronic cough (79%), and weight loss (71%) were the 3 most common presenting symptoms among PLHIV with definite and possible NTM disease. Patients in the definite NTM disease group have significantly lower CD4 than the 2 other groups. The most common abnormal chest x-ray findings were non-specific infiltrates followed by nodular densities. Nine patients were treated for NTM with most patients also managed for TB presumptively and empirically. There were 2 deaths in the definite NTM disease group, both with M. avium isolates.*

Introduction

The emergence of Human Immunodeficiency Virus and Acquired Immune Deficiency Syndrome (HIV/AIDS) as an epidemic has changed the epidemiologic landscape of several other infectious diseases including those caused by mycobacteria such as tuberculosis (TB) and nontuberculous mycobacteria (NTM). For example, the rapid spread of the HIV epidemic in the sub-Saharan Africa triggered an increase of almost five times in the TB notification rates between 1990 and 2005 [1].

TB is the most common presenting illness and cause of mortality among HIV-infected individuals [2]. In local studies, despite the decrease in prevalence brought by the increased use of antiretroviral drugs (ARV), TB remains as the most common opportunistic infection among people living with HIV (PLHIV) with a prevalence rate of 10.7% [3,4]. Compared to those without HIV infection, diagnosing TB among PLHIV is more difficult because patients can present with minimal to absent symptoms, and many can have atypical chest x-ray [5]. This TB and HIV/AIDS co-infection can be further complicated by the emergence of NTM which have overlapping features with MTB but with a different therapeutic approach [6].

Nontuberculous mycobacteria, also known as atypical mycobacteria or mycobacteria other than tuberculosis (MOTT) refer to all species of mycobacteria except those of *Mycobacterium tuberculosis* complex (MTBC), and *Mycobacterium leprae*. They are saprophytic and free-living environmental organisms commonly found in soil and various water sources [7-9]. More than 160 NTM species have been described in the literature but an overwhelming majority are not or rarely isolated clinically [10-12]. Some of these NTM species usually cause disease in patients with immunodeficiency or a pre-existing lung disease [7,13]. The incidence of NTM isolation and disease globally is rising in the last 2 to 3 decades due to improvements in diagnostic technology and the emergence of HIV/AIDS. However, its epidemiologic evaluation continues to be a huge challenge because unlike TB, NTM disease is not a reportable condition in most countries including the Philippines and it is rarely transmitted from human-to-human [8-11,14-16].

Since NTM are ubiquitous in the environment and humans are commonly exposed to it, these confounded the diagnosis of NTM disease. Not like TB, isolation of NTM in a nonsterile specimen like sputum may not always be clinically relevant because such isolation can also be due to contamination or colonization [8,15]. Because of the increasing frequency of NTM and its accompanying diagnostic challenges, the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) have published a guideline for the diagnosis and management of NTM in 1997 and updated it in 2007. The guideline divided NTM disease into four clinical syndromes namely: pulmonary disease, lymphadenitis, cutaneous disease and disseminated disease [8]. The pulmonary disease is the most common NTM syndrome while the disseminated disease is usually seen among severely immunocompromised patients such as PLHIV with CD4 level ≤ 50 cells/mL [8-10,17]. For the NTM pulmonary disease, the guideline specified strict clinical, radiologic and microbiologic criteria that should be met to avoid unnecessary treatment of patients with expensive and potentially toxic drugs. See Appendix A [8]. However, the guideline also conceded that it is limited in evaluating the significance of pulmonary NTM in resource-limited settings and in HIV-positive individuals.

Nontuberculous mycobacteria have been widely documented in developed and low TB-burden countries but less is known about the clinical importance of NTM among PLHIV in developing and high TB-burden countries like the Philippines [8-10,14-16]. Due to the high prevalence of MTB among PLHIV, the diagnosis of NTM disease is challenging in this specific population. It is often misdiagnosed or underdiagnosed due to overlap with TB disease, absence of awareness, deficient laboratory facilities and overburden of other diseases [17,18]. In countries like the Philippines where TB is highly prevalent and a serious public health problem, an HIV-infected patient who presents with chronic cough and/or fever and weight loss and has sputum test that is acid-fast bacilli (AFB) smear-positive or has chest x-ray (CXR) findings suggestive of TB, is generally presumed to have pulmonary TB and is treated empirically with anti-TB drugs [9,19]. Misdiagnosing NTM disease as TB can cause significant morbidity and mortality among PLHIV since treatment is not the same.

Studies in Kenya, Ghana, Colombia, China, and India have reported NTM pulmonary disease among PLHIV with varying frequency of isolation and prevalence rate [20-24]. In a study done in 3 countries in Southeast Asia where the Philippines is geographically located, it was reported that the overall prevalence of NTM disease in the HIV population was 2 percent [9]. Locally, there are limited studies about NTM, especially among PLHIV. In the study of Montoya, *et al.* in 1998, they isolated only 3 NTM species among 80 HIV-positive patients suspected of having pulmonary TB [25]. In a more recent study about opportunistic infections in patients with HIV, they only identified 4 NTM infections out of the 1549 PLHIV [4]. However, both local studies did not describe the patients' clinical profile nor the clinical significance of the isolated NTM. Another local study reported the isolation of NTM in 157 out of 6886 various specimens from the general population submitted for mycobacterial cultures; but clinical correlation was also lacking [26]. To increase the awareness of clinicians and recognize NTM as a possible cause of this clinically relevant disease, the present study will provide data regarding the clinical, radiologic and microbiologic characteristics of NTM infections among PLHIV. To the best of our knowledge, this is the first local study describing the NTM clinical profile among HIV–infected individuals.

Research Question

What are the clinical, radiologic and microbiologic features of nontuberculous mycobacterial diseases among people living with HIV (PLHIV) in the University of the Philippines -Philippine General Hospital (UP-PGH)?

Objectives

General Objective

To describe the common nontuberculous mycobacterial infections among PLHIV at the University of the Philippines - Philippine General Hospital.

Specific Objectives

1) To identify the common NTM species and NTM diseases found among PLHIV in a tertiary government hospital
2) To describe the clinical profile and outcomes of PLHIV with NTM diseases
3) To determine the antibiotic susceptibility of the identified NTM species

Methodology

Study Setting

This descriptive study was conducted in the University of the Philippines – Philippine General Hospital (UP-PGH), a 1500-bed tertiary government hospital in Manila, Philippines. UP-PGH is the largest hospital in the Philippines and the designated National University Hospital. It has more than 1000 service beds and 500 pay beds for private patients [27]. In 1997, to cater to the growing numbers of PLHIV and to address the rising hesitancy among healthcare workers in handling such cases, the hospital established the STDs and AIDS Guidance, Intervention and Prevention Unit or SAGIP Unit [28]. It is an HIV/AIDS clinic that offers both outpatient and in-patient services including HIV counselling, testing, clinical care and antiretroviral drugs. The SAGIP Clinic is the 3rd largest HIV treatment hub in the country in terms of patient enrollment.

Study Population

The study included enrolled and newly-enrolled PLHIV in the SAGIP clinic including those admitted in the service and pay wards of UP-PGH. Patients should be ≥ 19 years old and had clinical specimens which grew NTM in their mycobacterial cultures submitted between November 1, 2013 and January 31, 2016 to either of the 2 laboratories in UP-PGH - the Central Laboratory and/or Medical Research Laboratory (MRL). Excluded were patients with missing culture specimens and/or with missing or markedly incomplete medical records.

Newly-enrolled PLHIV in SAGIP, regardless of presence or absence of symptoms, were routinely screened for TB which included chest x-ray, sputum mycobacterial smear and culture along with other baseline laboratory examinations during their initial consult.

General Procedures and Data Collection

The census of the Section of Infectious Diseases and the logbooks of the Microbiology Section of the Central Laboratory and the TB Laboratory of MRL were reviewed for PLHIV. Likewise, census data of patients from the SAGIP clinic and hospital wards who submitted specimens for mycobacterial cultures were gathered. The Central Laboratory utilized only Mycobacterial Growth Indicator Tubes (MGIT) in its mycobacterial cultures while the MRL used both MB/Bact detection system and Ogawa medium. Both laboratories used MPT64 to differentiate the cultures with positive mycobacterial growth into MTB or NTM but neither could speciate the NTM isolates.

Available cultures of HIV-infected patients which grew NTM were retrieved from the 2 laboratories for speciation and drug susceptibility testing (DST) in the TB Laboratory of MRL. The NTM isolates were speciated using the REBA Myco-ID test kit. Then, the speciated NTM were subcultured for susceptibility testing.

The medical records of patients with speciated NTM isolates were obtained from the SAGIP clinic and the records section of the hospital. These were reviewed to obtain demographic and clinical information including environmental exposures, past medical and personal history, HIV treatment status, and other behavioral factors. Symptoms and signs present at the time when the cultures were done including, but not limited to cough and fever of at least two weeks, unintentional weight loss, night sweats, dyspnea, hemoptysis, and anorexia were recorded. Other clinical manifestations such as diarrhea, abdominal pain, easy fatigability and presence of lymph nodes were also noted. CD4 count, GeneXpert MTB/RIF results, chest x-ray findings and other imaging results were also gathered.

After the review of medical charts, the diagnosis of NTM disease among PLHIV were classified into 3 categories namely: 1) definite NTM disease (including pulmonary, extrapulmonary and disseminated); 2) possible NTM disease; and 3) not NTM disease [9]. The definitions for these categories are listed in Table 1. We have chosen to slightly modify and simplify the criteria of the 2007 ATS/IDSA statement for the classification of NTM pulmonary disease since our study was conducted in a resource-constraint setting with almost all patients unable to afford repeated mycobacterial cultures and additional imaging studies such as CT scan. In addition, HIV-positive patients are high-risk for mycobacterial morbidity and mortality and the rigorous criteria stated in the guideline might prevent or delay the timely diagnosis and appropriate management in clinical practice [8,9]. Thus, even if the ATS/IDSA guideline specified the isolation of NTM in at least 2 different sputum specimens for diagnosis, we have decided that growth of NTM in a single sputum specimen as sufficient in this study. This particular guideline likewise admitted its limitation in evaluating pulmonary NTM among PLHIV and its utility in resource-limited and TB-endemic settings has not been fully assessed.

Treatment and disease outcomes of the included PLHIV were also reviewed thereafter. Figure 1 illustrates the flow diagram for this study.

Laboratory Methods

A. Identification of NTM species

Specimens with NTM underwent REBA Myco-ID (M&D, Korea)

to identify the isolate up to species level. REBA Myco-ID is a molecular diagnostic kit that can detect and identify MTB and 19 species of NTM with Reverse Blot Hybridization Assay (REBA) by binding the amplifying *rpo*B gene product to species-specific probe [29].

- ***DNA Extraction***

The liquid medium with NTM was transferred in a sterile tube and was centrifuged at 12,000 rpm for 1 minute. Next, one pellet in the medium was re-suspended in 1 mL sterile distilled water and was centrifuged again. After repeating this step twice, the pellet was re-suspended in 100µL of DNA extraction solution (M&D, Korea) and was spun for another minute. The suspended bacterial solution was boiled for 10 minutes and was centrifuged at 13,000rpm for 3 minutes. A 3µL of the supernatant was used as DNA template for polymerase chain reaction (PCR) [29,30].

- ***PCR-REBA***

The REBA Myco-ID was performed according to the manufacturer's instructions [29]. Briefly, the PCR mixture was prepared using 20µL reaction mixture which contained 10µL 2X PCR Premix, 2 µL primer I, 3 µL of the DNA sample and 5µL sterile water. The PCR cycle constituted the pre-denaturation for 1 cycle, initially at 94^{0}C for 30 seconds followed by primer annealing and extension at 65^{0}C for 30 seconds each for 45 cycles. After one cycle, the samples were maintained at 72^{0}C for 7 minutes for completion strand synthesis. The amplified target was visualized as a single band corresponding to a length of 270 base pairs.

- ***Hybridization***

The amplified PCR products were then subjected to REBA. Fifteen microliter (15µL) of the PCR product was denatured with 15µL denaturation solution (1:1) and was incubated at 25^{0}C for 5 minutes. The membrane strip was adjusted into the blotting tray with hybridization solution. The denatured single-stranded DNA were mixed with 470 µL of hybridization solution and were hybridized at 50^{0}C, 90 rpm (orbital shaker bath) for 30 minutes while pre-warming the washing solution at 63^{0}C. The membranes were washed twice with the pre-warmed washing solution for 10 minutes at 90 rpm followed by the removal of the washing solution. One milliliter (1mL) of 1:2000 diluted streptavidin-alkaline phosphatase (AP) conjugate was added and incubated at 25^{0}C for 30 minutes at 90 rpm. The AP conjugate solution was removed and the membranes were washed with 1mL of conjugate diluent solution (CDS) at room temperature for 1 minute. A 1:50 diluted alkaline phosphatase mediated staining solution (NBT/BCIP, Roche Diagnostics) was added to visualize the colorimetric hybridization signals after 5 minutes incubation. The band patterns were read and interpreted using the REBA Myco-ID data sheet. The failure to hybridize maybe cause by insufficient DNA number or mismatched of nucleotides. Failure of the REBA Myco-ID test to identify the NTM species in the liquid culture can be due to presence of other bacteria in the culture such as *Nocardia* spp. or *Rhodococcus* spp.

Drug Susceptibility Testing (DST)

The NTM isolates were subcultured by inoculating approximately 0.1 - 0.2mL of a well-mixed positive 7H9 broth and incubated for one week. Growth was subjected to AFB stain to determine purity and was inoculated to Ogawa medium and incubated at 37^{0}C [31]. Inoculum was prepared by getting a loopful of growth from the Ogawa medium and transferring it to a 2.0mL sterile saline for rapidly-growing NTM and Middlebrook 7H9 broth for slowly-growing NTM. Inoculum was matched to 1.0 McFarland standard using a densitometer.

For rapidly-growing mycobacteria (RGM), antibiotic susceptibility testing was done using Etest strip (Biomerieux, France) [32]. The mycobacterial suspension was applied by swabbing the surface of a 150mm diameter plates of Muller-Hinton agar supplemented with 5% sheep's blood (BMHA). The Etest strips were placed on the plates and were incubated in ambient temperature at 35^{0}C - 37^{0}C. The minimum inhibitory concentration (MIC) was determined by the intersection of the inhibition ellipse with the concentration of antimicrobial agent on the Etest strip. The antibiotics and their susceptibility breakpoints used in this research were amikacin (≤16 µg/ml), cefoxitin (≤16 µg/ml), ciprofloxacin (≤1 µg/ml), clarithromycin (≤2 µg/ml), doxycycline (≤1 µg/ml), linezolid (≤8 µg/ml) co-trimoxazole (2/38 µg/ml) and imipenem (≤4 µg/ml) [31].

For slowly-growing mycobacteria (SGM), the method used for DST was the agar proportion method using Middlebrook 7H10 agar medium with OADC supplement. Critical drug concentrations used were as follows: 5.0 µg/mL for isoniazid, 1.0 µg/mL for rifampicin, 5.0 µg/mL for ethambutol, 10.0 µg/mL for streptomycin, 15.0 µg/mL for clarithromycin, 12.0 µg/mL for amikacin, 2.0 µg/mL for ciprofloxacin, 0.5/9.5 µg/mL for co-trimoxazole, and 6.0 µg/mL for doxycycline [31]. Inoculum was prepared as described above. A 10^{-2} and 10^{-4} dilution of the standardized suspension were prepared and inoculated separately into the control and the drug-containing quadrants. The plates were incubated at 35^{0}C -37ºC and drug susceptibility test results were read after 5 days with final reading after 10 days. Resistance ratio was computed by the number of colony forming units (CFUs) growing on the medium containing the drug compared with the number on the control plate.

Data Analysis

Microsoft Excel 2016 was used in encoding and deriving descriptive data. Descriptive statistics such as mean, median, standard deviation, range were computed from the quantitative data while categorical data were presented as numbers and percentages. SPSS 13.0 was used for statistical analysis. For two groups, the statistical tests used were Fisher's exact test for determining significant difference in percentages and Mann-Whitney U test for determining significant difference in CD4 count distribution. Meanwhile, Kruskall-Wallis test was used to determine significant difference CD4 count distribution for more than 2 groups. Differences were considered significant if p-value was less than 0.05.

Ethical Considerations

The research study was reviewed and approved by the UP-PGH Expanded Hospital Research Office (EHRO). Strict confidentiality

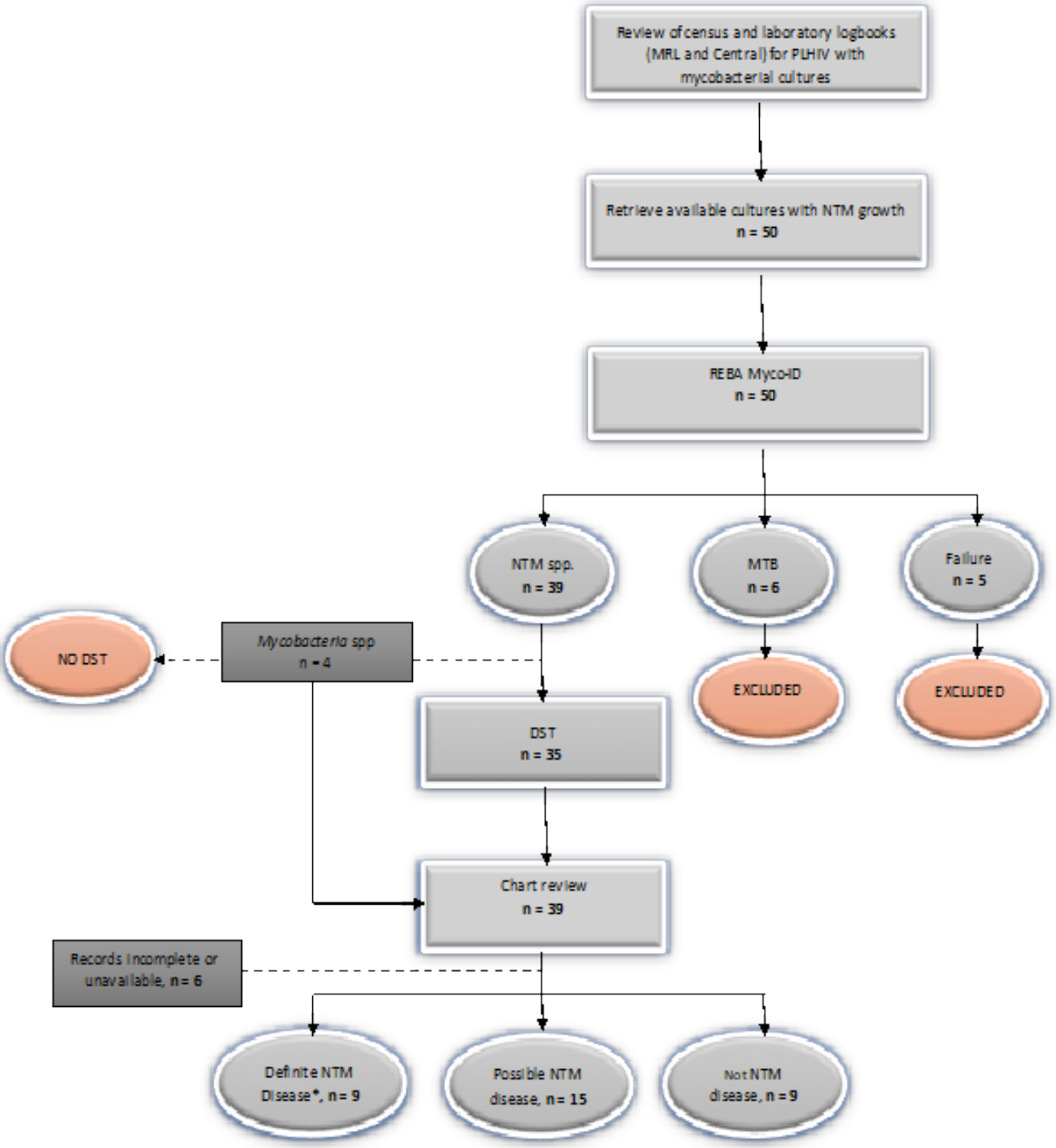

***Figure 1*: Flow Diagram.** NTM = nontuberculous mycobacteria; MTB = *Mycobacterium tuberculosis*; DST = drug susceptibility testing; MRL = Medical Research Laboratory; * includes both NTM pulmonary disease and extrapulmonary NTM disease

was observed as only the investigators were allowed to review the charts and each patient was assigned a code used in the data collection. The gathered data were encoded in a password-protected file to analyze the results. All medical charts of the included patients remained on site in UP-PGH with other patients' records. The completed data collection forms were kept in a secured cabinet of the primary investigator. A master list of patients with matching code numbers was kept in a password-protected file.

Table 1: Definitions of definite NTM disease, possible NTM disease and not NTM disease used in this study.

DEFINITE NTM DISEASE (including Pulmonary, Extrapulmonary and Disseminated)
A. Pulmonary Disease
• At least one pulmonary specimen with NTM growth, **WITH** • No respiratory specimen positive for MTB (culture and/or in GeneXpert) • Pulmonary symptoms such as cough, dyspnea or hemoptysis, **AND** • Chest x-ray findings like cavity, mass, nodule, bronchiectasis or infiltrates
B. Extrapulmonary NTM
• NTM isolated from a single location other than the lungs, blood or bone marrow, **AND** • Accompanying symptoms from the involved organ where NTM was isolated
C. Disseminated disease
• Isolation of same NTM species from ≥2 sites in the same patient, **OR** • Blood or bone marrow cultures positive for NTM
POSSIBLE NTM DISEASE (Pulmonary)
• At least one pulmonary specimen with NTM growth, **WITH** • No respiratory specimen positive for MTB (culture and/or in GeneXpert), **AND** • **EITHER** with pulmonary symptoms **OR** chest x-ray findings similar to mentioned above
NOT NTM DISEASE (Pulmonary)
• At least one pulmonary specimen with NTM growth, **WITH** • No respiratory specimen positive for MTB (culture and/or in GeneXpert), **AND** • No pulmonary symptoms **AND** • Normal chest x-ray findings

Abbreviations: NTM = nontuberculous mycobacteria; MTB = Mycobacteria tuberculosis

Source: McCarthy KD, Cain KP, Wintrop KL, Udomsantisuk U, Lan NTN, et al. Mycobacterial Disease in Patients in Patients with HIV. *Am J Respr Crit Care Med*. 2012;185(9): 982.

Results

Microbiologic Profile

Upon the review of logbooks in UP-PGH Central Laboratory and Medical Research Laboratory (MRL), there were 330 specimens from PLHIV submitted for mycobacterial cultures from November 1, 2013 to end of January 2016. Three-hundred and fifteen specimens were submitted to the Central Laboratory and 15 to the MRL. An overwhelming majority of specimens were obtained from the respiratory tract - sputum and endotracheal aspirate (82%); while, the rest came from other sources such as cerebrospinal fluid (6%); abscesses and aspirates (3%); stool samples (3%); tissues (3%), blood and bone marrow (1%), and other body fluids (2%). We retrieved 50 cultures with NTM growth from different specimens (46 from sputum, 2 from stool, 1 from tissue and 1 from abscess) for further testing in the TB Laboratory of MRL. Using REBA Myco-ID, we identified the specific species in 39 NTM isolates. However, 6 of the 50 NTM isolates turned out to be MTB on speciation (including 1 specimen mixed with *M. fortuitum*); while another 5 isolates had test failures and remained unidentified after REBA Myco-ID. Specimens with MTB isolates or had test failures were excluded.

Of the 39 cultures with speciated NTM, 26 contained single NTM specie, 9 had mixed NTM species and 4 samples were identified only as other *Mycobacteria* spp. The most common NTM specie in this study, regardless of clinical significance was *Mycobacterium fortuitum* which was isolated in 15 specimens, including 2 specimens which were mixed with other NTM species. This was followed by *Mycobacterium avium* identified from 9 cultures with 4 mixed with other NTM species. The third most common was *M. mucogenicum* from 5 cultures, 4 of which were also mixed with other NTM species. Other mycobacteria identified were *M. intracellulare* (3 - 2 were mixed), *M. terrae* (3 – all were mixed), *M. gordonae* (2 – both were mixed), *M. genavense*/M.simiae (2), *M. massiliense* (2), *M. scrofulaceum* (1), *M. aubagnense* (1), *M. abscessus* (1 – mixed). Table 2 enumerates the NTM species identified by the REBA Myco-ID.

Table 2: Mycobacterial species identified by REBA Myco-ID (N=39).

Rapidly Growing Mycobacteria	n=17	Slowly Growing Mycobacteria	n=9	Mixed Nontuberculous mycobacteria (NTM)	n=9
M. fortuitum	13	*M. avium*	5	*M. avium / M. terrae*	2
M. massiliense	2	*M. intracellulare*	1	*M. avium / M. mucogenicum*	1
M. mucogenicum	1	*M. scrofulaceum*	1	*M. avium / M. abscessus*	1
M. aubagnense	1	*M. genavense/ simiae*[a]	2	*M. fortuitum / M. mucogenicum*	1
				M. fortuitum / M. intracellulare	1
*Mycobacteria spp. = 4				*M. mucogenicum / M. intracellulare*	1
				M. mucogenicum / M. gordonae	1
				M. gordonae / M. terrae	1

[a] *both species use the same probe in REBA Myco-ID*

Only 36% (14/39) of the cultures were also AFB smear-positive. Majority of *M. fortuitum* isolated (9/15) was found in the definite and possible NTM disease groups, mostly in the latter group (8/9). Half of all isolates (6/12) of *M. avium* and *M. intracellulare*, collectively called *Mycobacterium avium* complex (MAC) were found among patients in the definite NTM disease category with 6 of the 9 individuals in this group having MAC as isolates. Known contaminants like *M. mucogenicum, M. gordonae* and *M. terrae* were mostly found in the not NTM disease group. Table 3 lists the NTM species found in each disease category after identification using REBA Myco-ID. Figure 2 are photographs of the actual REBA Myco-ID conducted in some of the mycobacterial isolates.

Table 3: Mycobacterial species identified by REBA Myco-ID and classified according to NTM disease category (N=39).

	Total (N = 39)	Definite NTM Disease (n = 9)	Possible NTM Disease (n = 15)	No NTM Disease (n = 9)	PLHIV with No records (n = 6)
AFB smear positive	14	5	3	2	4
M. fortuitum	14 [b]	1 [b]	7	5	1
M. avium	9 [b, c, f]	5 [a, b]	3 [a]	0	1 [c]
M. intracellulare	3 [b, e]	1	1 [e]	1 [b]	0
M. mucogenicum	2 [d]	0	2 [d]	0	0
M. genavense/simiae	2	0	1	0	1
M. massiliense	2	0	1	0	1
M. scrofulaceum	1	0	0	1	0
M. terrae	1 [d]	0	0	1 [d]	0
M. aubagnense	1	1	0	0	0
Mycobacteria spp.	4	1	0	1	2

Abbreviations: NTM, nontuberculous mycobacteria; AFB, acid-fast bacilli; PLHIV, people living with human immunodeficiency virus

[a] *1 isolate mixed with M. terrae . .*

[b] *1 isolate mixed with M. mucogenicum.*

[c] *1 isolate mixed with M. abscessus*

[d] *1 isolate mixed with M. gordonae*

[e] *1 isolate mixed with M. fortuitum*

[f] *2 isolates mixed with M. terrae*

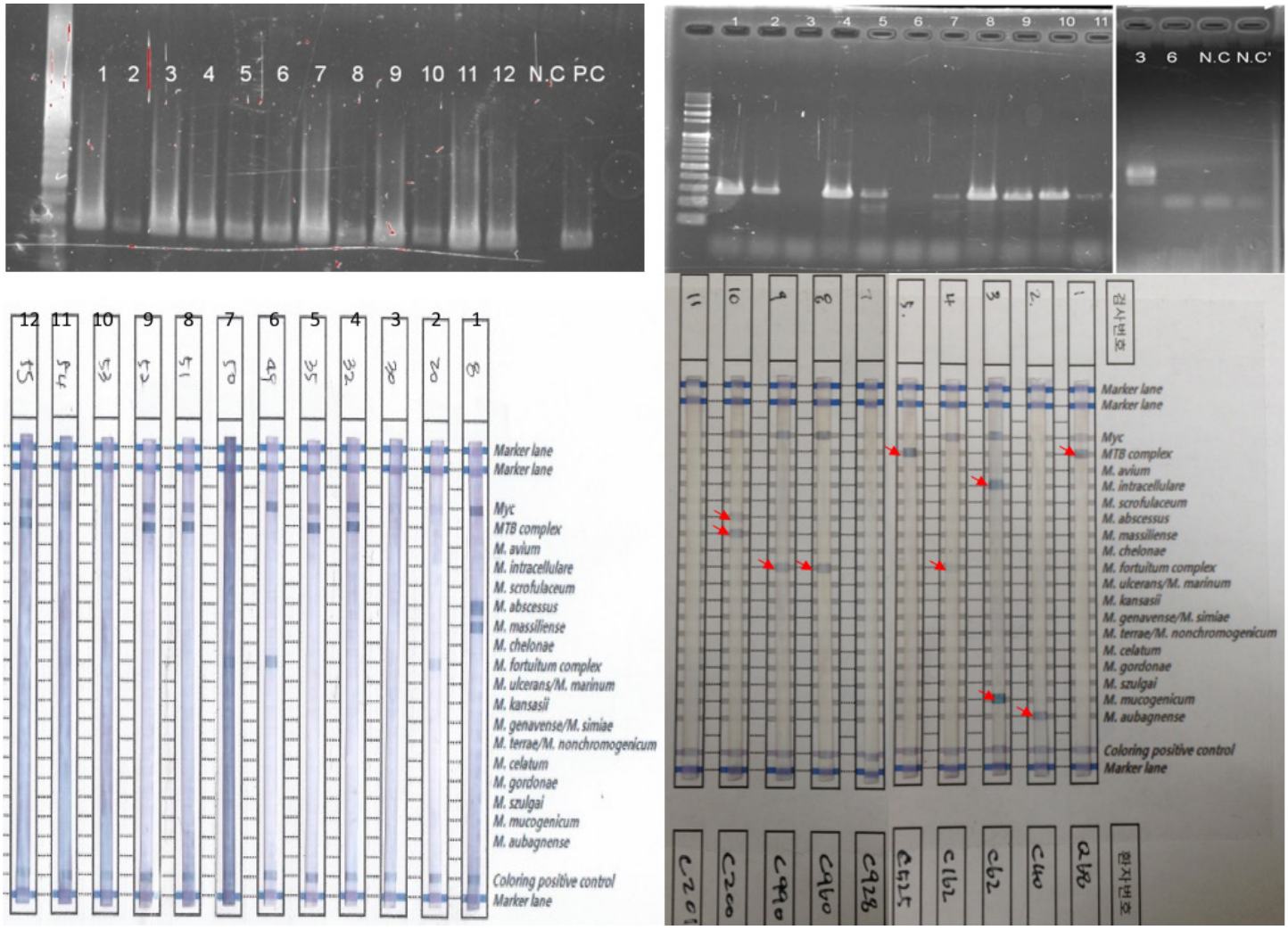

Figure 2: Actual REBA Myco-ID test of the mycobacterial Isolates.

Mycobacterial isolates determined by REBA Myco ID: LEFT – Lane 1: M. massiliense; Lane 2: M. fortuitum; Lane 3: Other bacteria; Lane 4: MTB; Lane 5: MTB; Lane 6: M. fortuitum; Lane 7: M. fortuitum; Lane 8: MTB; Lane 9: MTB; Lane 10: Other bacteria; Lane 11: Mycobacteria spp. Lane 12: MTB. RIGHT - Lane 1: MTB; Lane 2: M. aubagnense; Lane 3: M. intracellulare/M. mucogenicum mix; Lane 4: M. fortuitum; Lane 5: MTB; Lane 7: Other bacteria; Lane 8: M. fortuitum; Lane 9 M. fortuitum; Lane 10: M. massiliense; Lane 11: Other bacteria. Data interpretation of M. abscessus only show positive band pattern for M. abscessus probe and M. massiliense shows positive band patterns for M. abscessus and M. massilience probe.

Drug Susceptibility Testing

Thirty-five cultures with speciated NTM underwent DST, regardless of the clinical significance of the NTM species. The 4 isolates identified as other *Mycobacteria* spp. by the REBA Myco-ID were not tested for drug susceptibility. There were 18 cultures with rapidly-growing mycobacteria (17 with single RGM isolate and 1 with mixed culture of 2 RGM), 12 with slowly- growing mycobacteria (9 single SGM and 3 with mixed cultures of 2 SGM) and 5 cultures with mixture of 1 slow-grower and 1 rapid-grower. The rapid-growers isolated were *M. fortuitum, M. massiliense, M. mucogenicum, M. aubagnense*, and *M. abscessus* while the slow

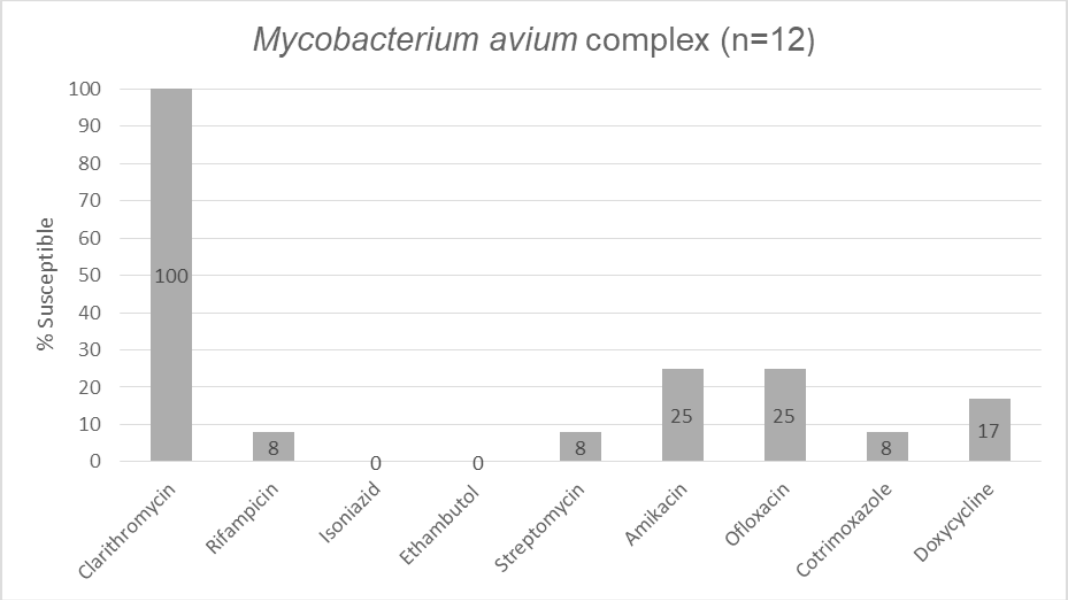

Figure 3: Drug susceptibility of *Mycobacterium avium* complex (MAC).

M. fortuitum was found in 15 cultures, including 2 which were mixed with other NTM species. *M. fortuitum* was 100% sensitive to both ciprofloxacin and amikacin. Of the 15 isolates, only 1, 3, 5, 9, and 10 isolates were susceptible to linezolid, imipenem, clarithromycin, cefoxitin, doxycycline, respectively. Figure 4 illustrates the drug susceptibility of *M. fortuitum.*

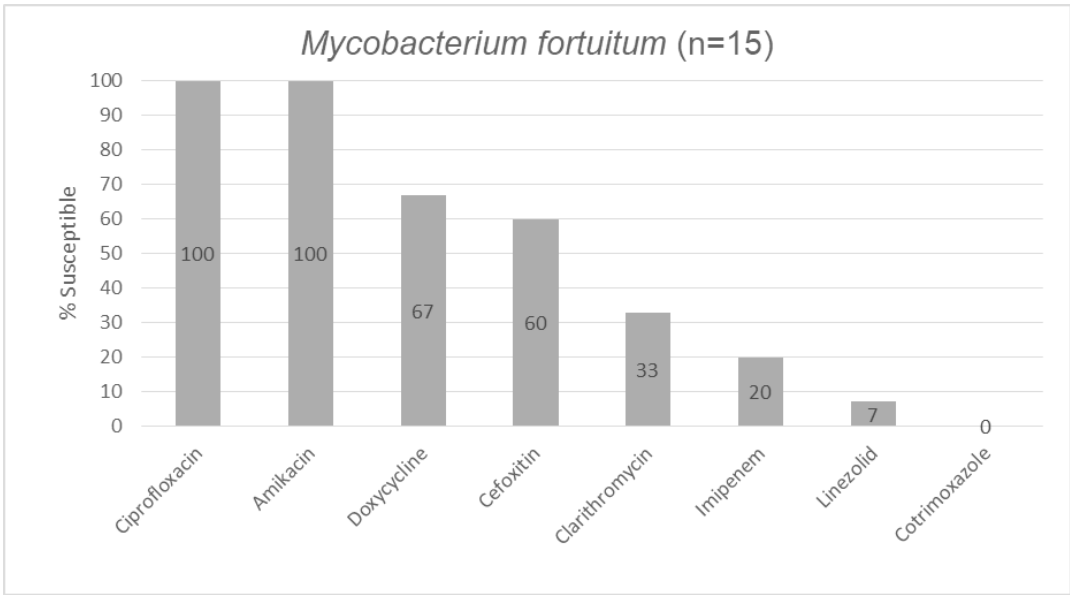

Figure 4: Drug susceptibility of *Mycobacterium fortuitum.*

growers were *M. avium, M. intracellulare, M. scrofulaceum, M. genavense/simiae, M. terrae,* and *M. gordonae*. See Table 2. We gave importance to the DST of *Mycobacterium avium* complex (MAC) since it is the most common NTM species isolated among PLHIV in most literature [20-24,34,46]. Another susceptibility graph was created for the RGM *M. fortuitum* since it was the most common NTM specie isolated in this study. The antibiotic susceptibilities of other NTM species can be seen in the Appendix E. Twelve cultures had MAC: 9 with *M. avium* and 3 with *M. intracellulare*. Single species of *M. avium* and *M. intracellulare* were isolated from 6 cultures while another 6 had *M. avium* or *M. intracellulare* mixed with other NTM species. All 12 isolates of MAC were susceptible to clarithromycin but were resistant to ethambutol and isoniazid. Eleven of the twelve MAC isolates were also resistant to rifampicin and streptomycin while 9 were also not sensitive to amikacin. Figure 3 illustrates the drug susceptibility of MAC.

Clinical Profile

We reviewed the medical records of the 33 out of the 39 PLHIV with speciated NTM. Six charts had markedly incomplete clinical details since these were private patients referred only to SAGIP for the supply of their antiretroviral drugs. After the chart review, the 33 HIV-infected individuals were categorized into having definite NTM disease with 9 cases (8 pulmonary NTM and 1 extrapulmonary NTM), possible NTM disease with 15 cases, and not NTM disease with 9 cases. Based on the criteria used in this study, the patients in the not NTM disease category were expectedly asymptomatic and had normal chest x-ray findings thus, we did not include them in the description and analysis of clinical profile.

The twenty-four patients from the definite and possible NTM disease groups were all males with a mean age of 30.0 ± 8.4 years old. All but one PLHIV resided in the urban and suburban areas. Majority (11/24) were employed in offices such as call center agents, sales specialists, and clerks, while the rest worked as teachers (2), seafarer (1), engineer (1), flight attendant (1), and healthcare worker (1). There were 4 unemployed, 2 students and 1 with missing occupation data. Most were smokers (58%) and alcoholic beverage drinkers (88%). Except for a single chart which stated a history of farming in a patient with possible NTM disease, all records lacked data about environmental exposures including water source. Twenty patients (83%) were newly-diagnosed with HIV infection, thus were all treatment-naïve; while 4 patients were known HIV cases and were already taking antiretroviral drugs when NTM were isolated.

At the time when their mycobacterial cultures were obtained, half of the 24 cases had no past or current TB treatment while 7 (29%) and 5 (21%) patients, respectively, reported a previous and ongoing TB medications. Except for pulmonary TB, only one patient reported a history of chronic pulmonary disease (bronchial asthma). All PLHIV had no history of diabetes mellitus (DM).

The PLHIV in the two NTM categories reported both pulmonary and non-pulmonary symptoms. A large majority (19/24) reported cough with individuals from the definite NTM group having a longer mean cough duration (7.3 ± 3.0 vs. 4.8 ± 2.6 weeks) than those from the possible NTM group but with no statistical difference (p = 0.292). Except for the single patient with extrapulmonary NTM disease, all patients with definite NTM disease presented with cough. Dyspnea (3/24) was uncommon while hemoptysis was absent in all patients. Table 4 describes the demographic and clinical features of the included patients.

Table 4: Demographic and clinical features of PLHIV with definite and possible NTM disease (n = 24).

Clinical characteristics	Total (n = 24)	Definite NTM Disease [a] (n = 9)	Possible NTM Disease (n = 15)	*p*-value[d]
Age, Mean ± SD (range, *in years*)	30.0 ± 8.4 (19-57)	29.1 ± 6.7 (20-37)	30.5 ± 9.5 (19-57)	0.698
Sex (M:F)	24:0	9:0	15:0	-
Anti-retroviral drugs				
Ongoing treatment (%)	4 (17)	4 (44)	0	0.012
Treatment-naïve (%)	20 (83)	5 (56)	15 (100)	
Tuberculosis history				
Past TB treatment (%)	7 (29)	4 (44)	3 (20)	0.387
Ongoing TB treatment (%)	5 (21)	1 (11)	4 (27)	
No history of TB treatment (%)	12 (50)	4 (44)	8 (53)	
Smoker (%)	14 (58)	6 (67)	8 (53)	0.678
Alcoholic beverage drinker %)	21 (88)	7 (78)	14 (93)	0.553
Drug user (%)	8[b] (35)	3 (33)	5[b] (36)	1.000
Any Cough (%)	19 (79)	8 (89)	11 (73)	0.614
≥ 2 weeks but < 4 weeks (%)	5 (21)	1 (11)	4 (27)	0.338
≥ 4 weeks	14 (58)	7 (78)	7 (47)	
Hemoptysis (%)	0	0	0	-
Dyspnea (%)	3 (13)	2 (22)	1 (7)	0.533
Fever (%)	19 (79)	8 (89)	11(73)	0.615
≥ 2 weeks but < 4 weeks (%)	13 (54)	4 (44)	9 (60)	0.319
≥ 4 weeks but < 8 weeks (%)	6 (25)	4 (44)	2 (13)	
Weight loss (%)	17 (71)	8 (89)	9 (60)	0.191
Anorexia ≥ 2 weeks (%)	7 (29)	4 (44)	3 (20)	0.356
Night sweat ≥ 2 weeks (%)	4 (17)	2 (22)	2 (13)	0.615
Diarrhea (%)	5 (21)	4 (44)	1 (7)	0.047
Abdominal pain (%)	2 (8)	2 (22)	0 (0)	0.130
Easy fatigability (%)	2 (8)	0 (0)	2 (13)	0.511
Lymphadenopathy (%)	14 (58)	6 (67)	8 (53)	0.678
Cervical (%)	11 (46)	5 (56)	6 (40)	0.756
Inguinal (%)	3 (13)	1 (11)	2 (13)	
Mean weight ± SD, *kilograms* (range)	57.1[c] ± 11.1 (33.4-78)	59.0[c] ± 11.5 (42.2–78)	56.2 ± 11.1 (33.4-68.5)	0.680
Opportunistic infections / HIV-associated conditions				
PCP (%)	8 (33)	7 (78)	1 (7)	<0.001
Oral thrush (%)	13 (54)	7 (78)	6 (40)	0.105
Malignancy (%)	2 (8)	2 (22)	0 (0)	0.130
Recurrent carbuncles (%)	1 (4)	0 (0)	1 (7)	0.500

**column percentages*

Abbreviations: HIV, human immunodeficiency virus; PLHIV, people living with HIV; NTM, nontuberculous mycobacteria; PCP, Pneumocystis jirovecii pneumonia; SD, standard deviation

[a] includes 8 pulmonary and 1 extrapulmonary NTM; [b] 1 patient with missing data; [c] 2 patients with missing data ; [d] Fisher's exact test was used for comparing counts; Independent-samples Mann-Whitney U Test was used for comparing means/medians.

The main constitutional symptoms present among members of both groups were fever and weight loss. Almost all patients (8/9) in the definite NTM disease reported fever and weight loss while a majority complained of fever (11/15) and weight loss (9/15) in the possible NTM disease. Amount of weight loss was not quantified. Other symptoms like anorexia, night sweat, diarrhea, abdominal pain and easy fatigability were reported but not common. The only patient with an extrapulmonary NTM (gastrointestinal tract NTM) initially presented with abdominal pain and diarrhea with accompanying fever and weight loss. Lymphadenopathy, mainly cervical was also commonly seen in both groups. The PLHIV in the definite NTM group were slightly heavier than their counterparts in the possible NTM group but this was not statistically significant ($p = 0.680$).

History of opportunistic infections aside from tuberculosis, either current or previous, were frequently described in both groups. Oral thrush (13/24) was the most common followed by *Pneumocystis jirovecii* pneumonia or PCP (8/24). Seven of the eight reported cases of PCP were found in patients from the definite NTM group and only 1 case in the other group ($p = <0.001$). There were also 2 PLHIV in the first group with malignancies (Kaposi's sarcoma and lymphoma).

PLHIV in the not NTM disease group also reported a few constitutional symptoms such as fever (1/9), weight loss (3/9), and anorexia (1/9). Opportunistic infections were also observed in some patients (1 PCP and 2 oral thrush).

We included the CD4 count from patients in the not NTM disease category in the analysis and compared it to the CD4 count of those in the definite and possible NTM disease categories. Patients in the definite NTM disease had the lowest CD4 count with a median of 67 cells/mm^3 and was statistically significant (p = 0.032) compared to 294 cells/mm^3 and 369 cells/mm^3 in the possible and not NTM disease groups, respectively. Five of the nine PLHIV in the definite disease category had CD4 less than 100 cells/mm^3, including the 3 with less than 50 cells/mm^3. More than half (8/15) had CD4 level of more than 200 cells/mm^3 in the possible NTM disease group. Table 5 tabulates the CD4 counts in the 3 NTM disease categories.

Table 5: CD4 counts of PLHIV with definite NTM disease, possible NTM disease and not NTM disease (n = 32).

CD4 Count, *in cells/mm^3*	Total (n = 32) [b]	Definite NTM Disease [a, b] (n = 8)	Possible NTM Disease (n = 15)	Not NTM Disease (n = 9)	*p*-value [c]
CD4 count, median	210	67	294	369	0.032 *
CD4 < 100	13	5	7	1	0.107
CD4 100 - 200	3	1	0	2	
CD4 > 200	16	2	8	6	

Abbreviations: PLHIV, People living with HIV; NTM, nontuberculous mycobacteria

[a] includes 7 pulmonary and 1 extrapulmonary NTM; [b] 1 patient had missing data on CD4 count; [c] Independent-samples Kruskal-Wallis test was used

**Significant at p-value ≤ 0.05*

Presence of non-specific infiltrates was the most common chest x-ray abnormality and was seen in all eight patients with definite NTM pulmonary disease. An additional finding of nodular densities was also reported in 2 patients. The PLHIV with non-pulmonary NTM (gastrointestinal NTM) disease had a normal chest x-ray but his CT scan showed mediastinal and mesenteric lymphadenopathies. In contrast, most of the individuals (11/15) in the possible NTM disease category had normal chest x-ray. Only 3 and 1 patient/s had infiltrates and nodules, respectively in their chest radiographs. Cavitations and bronchiectasis were not reported in both groups. Table 6 summarizes the radiologic features of the PLHIV in the 2 groups.

Table 6: Chest radiographic findings of PLHIV with definite NTM disease and possible NTM disease (n = 24).

Chest x-ray findings	Total (n = 24)	Definite NTM Disease [a] (n = 9)	Possible NTM Disease (n = 15)	*p*-value
Normal	12 (50)	1 [c] (11)	11 (73)	0.009*
Infiltrates	11 (46)	8 (89)	3 (20)	0.002*
Masses/Nodules	3 (13)	2 [b] (22)	1 (7)	0.533
Cavitations	0	0	0	-
Bronchiectasis	0	0	0	-

column percentages

Abbreviations: PLHIV, People living with HIV; NTM, nontuberculous mycobacteria

[a] includes 8 pulmonary and 1 extrapulmonary NTM.

[b] 2 patients with additional chest x-ray findings

[c] extrapulmonary NTM (gastrointestinal NTM)

**significant at p-value ≤ 0.05*

Clinical Outcomes

Of the 9 PLHIV in the definite NTM disease group, only 5 were treated for NTM including the patient with extrapulmonary NTM. Four of these patients were initially managed for TB but were subsequently treated for NTM when there was minimal clinical response, or their culture results later revealed NTM growth and a single patient was treated simultaneously for TB and NTM pending the culture results due to critical medical condition. Mycobacterial cultures from these 5 patients revealed *M. avium* [4] and *M. aubagnense* [1]. Three PLHIV completed their TB and NTM treatment with full clinical improvement whereas, the other 2 patients treated for NTM died during the course of their treatment, both with *M. avium*.

Two of the 9 PLHIV from the definite NTM disease class were not treated for NTM and received only anti-Koch's drugs with good clinical outcome. NTM isolates in these 2 patients were *M. intracellulare* and *Mycobacteria* spp. Another 2 patients had unknown outcomes because one did not follow-up after the first consult and the other eventually transferred to a private physician.

In the possible NTM disease category, only 3 of the 15 PLHIV were treated for NTM: 2 patients were initially managed as TB but were later given NTM regimen due to minimal improvement while, one patient was treated for NTM only after he developed prominent cervical lymphadenopathy. Isolated from these 3 PLHIV were *M.*

fortuitum [2] and *M. genavense* [1]. Two patients completed their treatment with improved outcomes, while the other patient had unknown outcome after he transferred to another treatment hub during his treatment course.

Despite NTM isolation in their cultures, 6 of the 15 patients in the possible NTM disease group had clinical improvement with tuberculosis treatment only. NTM identified in these 6 patients were *M. avium, M. intracellulare, M. massiliense, M. mucogenicum* and *M. fortuitum*. A single patient from this possible NTM group did not receive TB nor NTM treatment but had no clinical deterioration. Five patients in this group had unknown management and outcomes due to lost to follow-up [4] or transfer to another treatment hub [1]. Table 7 describes the management and outcomes of the HIV-positive patients included in the study.

In the not NTM disease group, all 9 patients were not treated for NTM but one patient was given TB treatment. There was no clinical deterioration in all patients in this group.

It is also notable that PLHIV treated for NTM in both definite and possible NTM disease groups had lower CD4 counts than those who were not managed for NTM, although this was not statistically significant for the definite NTM disease group, as shown in Table 8.

Table 7: Management and outcomes of PLHIV with definite NTM disease and possible NTM disease (n = 24).

		Definite NTM Disease (n = 9) [g]			**Possible NTM Disease (n = 15)** [h]		
		Improved	**Died**	**Unknown**	**Improved**	**Died**	**Unknown**
Treated for NTM	**Treated for NTM only**	0	0	0	1 *(M. fortuitum)*	0	0
	Treated for TB and NTM	3 *(M. avium* [b] - 2 *M. aubagnense* -1)	2 *(M. avium* [a] *- 2)*	0	1 *(M. fortuitum)*	0	1 *(M. genavense)*
Not treated for NTM	**Treated for TB only**	2 *(M. intra-celulare - 1, Myco spp. – 1)*	0	0	6 *(M.mucogenicum* [d] *- 2, M. massiliense – 1, M. fortuitum – 1, M.intracellulare* [e] *– 1, M. avium* [a] *– 1)*	0	0
	Not treated for TB and NTM	0	0	0	1 *(M. fortuitum)*	0	0

Abbreviations: TB, tuberculosis; NTM, nontuberculous mycobacteria
Inside the parenthesis are the NTM Isolated
[a] *1 isolate mixed with M. terrae*
[b] *1 isolate mixed with M. mucogenicum.*
[c] *1 isolate mixed with M. abscessus*
[d] *1 isolate mixed with M. gordonae*
[e] *1 isolate mixed with M. fortuitum*
[f] *2 isolates mixed with M. terrae*
[g] *2 were lost to follow-up and had unknown outcome*
[h] *5 were lost to follow-up and had unknown outcome*

Table 8: Comparison of CD4 counts of PLHIV treated and not treated for NTM.

	Treated for NTM						***p*-value**
	Yes			**No**			
	No.	Mean (Median)	Range	No.	Mean (Median)	Range	
Definite NTM Disease	4[a]	32.5 (21)	14 - 74	2	210.5 (210.5)	165 - 256	0.133
Possible NTM Disease	3	47.3 (31)	23 - 88	7	341.9 (331)	41 - 502	0.033*

Abbreviations: PLHIV, People living with HIV; NTM, nontuberculous mycobacteria
Excluded were PLHIV who were lost to follow-up or transferred to another treatment hub
[a] *5 patients were treated for NTM but 1 had missing data on CD4*
**Significant at p-value≤ 0.05*

Table 9: Drug regimens used for treatment of nontuberculous mycobacteria (n = 8).

Drug regimens	**n**
Rifampicin + Ethambutol + azithromycin	6
Rifampicin + Ethambutol + clarithromycin	1
Azithromycin only	1

Treatment of NTM

The most common treatment regimen given to patients managed as NTM were macrolide plus rifampicin and ethambutol; the last 2 medications were part of the initial TB treatment and continued with the macrolide for 1 year. This regimen was used in 7 of the 8 PLHIV treated for NTM while one patient received azithromycin only. Azithromycin was the preferred macrolide over clarithromycin in 7 of the 8 patients who were treated for NTM. It should be noted that all treatment for NTM in this study were given without the benefit of speciation and drug susceptibility studies. Table 9 summarizes the NTM treatment regimens used in this study.

Discussion

Our study tried to characterize the clinical, radiologic and microbiologic features of NTM disease among PLHIV as we aspire to help clinicians in diagnosing this opportunistic infection and enabling them to provide prompt and proper management. However, as also described in other studies, the clinical features of NTM are non-specific, variable, and often indistinguishable with those of MTB [8-9,33-38]. The predominant symptoms of patients with NTM in this study were chronic cough, prolonged fever and weight loss which can also be the same symptomatology in TB [39]. These symptoms were also reported in previous studies of NTM disease involving both non-HIV-infected [7] and HIV-infected population [9,21]. Furthermore, HIV/AIDS can also manifest with the same constitutional symptoms such as fever, weight loss, anorexia. The cough, hemoptysis, and dyspnea can also be possibly due to another underlying lung disease or another opportunistic infection such as PCP.

NTM pulmonary disease in the general population usually follows radiographic patterns of either fibro-cavitary lesions which is similar to TB or the nodular/bronchiectatic disease; but these may not be true for HIV-positive individuals [8,35]. Similar to our results, a study involving PLHIV with definite NTM pulmonary disease have interstitial infiltrates as the most frequent pathological lesions in their chest radiographs. Also, none has bronchiectatic lesions in that study [34]. In another study of McCarthy, *et al.*, majority of PLHIV with NTM disease have nodules (67%), infiltrates (44%) and cavities (22%) in their chest x-rays [9]. It is also not unusual for PLHIV with possible or significant NTM to have a normal chest radiography, especially in the first few weeks of infection [34,40]. In our study, there were 2 patients from the possible NTM disease category who were subsequently treated for NTM despite having normal chest x-rays. The absence of radiographic evidence of pulmonary disease in PLHIV may indicate disseminated NTM disease or possibly a harbinger of disseminated disease. It can also suggest a transient infection [8]. The chest x-ray findings in NTM disease can also be dependent in the causative NTM species, for example, cavitations are more frequently associated with *M. abscessus* and *M. kansasii* [8].

As reported elsewhere, CD4 count was significantly lower among PLHIV with definite NTM disease against those with non-significant NTM. The CD4 also tends to be lower in HIV-infected patients with NTM disease compared to those with HIV and TB co-infection [9,21-22,34]. The level of CD4 may help clinicians determine the pathogenicity of the mycobacterial isolates. TB is typically diagnosed across a broad range of CD4 count levels, whereas NTM disease particularly disseminated disease occurs when CD4 cell counts are less than 50 cells/mm^3 [9]. However, in our study, there were 3 patients categorized as not NTM disease based in our study criteria but had CD4 level < 200 cells/mm^3 while, there were 2 individuals in the definite NTM disease group who had CD4 > 200 cells/mm^3. In these cases, determining clinical and pathogenic significance of the NTM isolates can be difficult and additional clinical, biochemical, and radiologic findings or closely monitoring the patients can be beneficial in making a treatment decision. The lower CD4 levels can also explain the presence of more frequent opportunistic infections among the PLHIV in the definite NTM disease group.

Of the 8 patients treated for NTM, seven were initially or simultaneously managed for TB before subsequently initiating NTM medications. Starting presumptive treatment for TB is a common practice in the Philippines where TB is endemic. Any patient with smear-positive specimens together with symptoms and radiographic findings suggestive of TB will be treated as such. Generally, this approach may be useful since TB is a lot more common than NTM in our country, but failure to properly recognize NTM especially among PLHIV can have devastating outcomes in this high-risk group [34,41]. Since our National TB Program is highly dependent on AFB smear findings for diagnosis of TB, many of these smear-positive NTM cases can be misdiagnosed as pulmonary TB.

Mycobacterium fortuitum and *Mycobacterium avium* complex (MAC) were the most common NTM isolates in our study. These NTM species are also among the top 5 most common in the world although geographic variation exists. *M. fortuitum* is highly prevalent in Asia particularly in East Asia where they account for 27% of all NTM isolates in Asia, while MAC is more common in Australia (71%) but can also be found with high prevalence in Japan [42]. Similar to the study of McCarthy, *M. fortuitum* was commonly isolated among patients classified as possible and not significant NTM disease [9]. Determining its clinical significance among PLHIV is tricky and difficult since it is known to be less pathogenic than other NTM species and sometimes a contaminant; thus, isolation from bronchopulmonary secretions may usually represent colonization than infection [8,43]. However, *M. fortuitum* among HIV-infected patients has been reported in literature causing clinically significant infections usually in lymph nodes, soft tissue, brain, and sometimes in the lungs hence, these patients must be continually assessed and monitored for development of significant disease [9,44-45]. In our study, only 2 of the 15 patients with *M. fortuitum* isolates were symptomatic and were treated with a macrolide-based regimen.

Unlike *M. fortuitum*, MAC when isolated is usually clinically significant especially among PLHIV. MAC is also the most

common NTM isolate associated with HIV/AIDS as reported in several studies [20-24,34,46]. In clinical practice, the *M. avium* and *M. intracellulare* are usually reported as MAC since separating it into 2 species does not affect treatment and prognosis of the NTM disease. An overwhelming majority of disseminated NTM infection in HIV is caused by *M. avium* while *M. intracellulare* is usually responsible for most MAC lung diseases [8,47]. As shown in our study, 67% of patients in the definite NTM disease category have MAC isolates (specifically *M. avium*). However, only 4 of the 12 patients with MAC isolates were treated for NTM, while 3 improved with TB medications only, 1 patient was neither treated for TB nor NTM, 3 were lost to follow-up, and 1 had missing medical record. It is likely that the isolated MAC in the 4 patients managed as TB or was untreated may represent colonization [46] since immunodepression in these patients were not severe as evidenced by their relatively high CD4 levels (mean = 356 cells/mm^3) coupled by the subsequent introduction of ARV in these patients. Another possibility is that standard anti-TB drugs such as ethambutol and rifampicin maybe effective *in vivo* against MAC despite its *in-vitro* resistance.

In Table 10, we tried to emphasize the clinical significance of MAC in HIV-infected individuals by comparing PLHIV with MAC isolates to those with NTM species other than MAC. PLHIV with MAC may be more symptomatic, have lower median CD4, more abnormal chest x-ray findings and more likely to be treated for NTM than those with other NTM species; but these are all not statistically relevant. Patients with MAC also had poorer outcome with 2 deaths in the group.

Table 10: Comparison of PLHIV with *Mycobacterium avium* complex (MAC) and PLHIV with NTM species other than *Mycobacterium avium* Complex

	PLHIV with MAC (n = 11)	PLHIV with NTM species other than MAC (n=22)	*p-value*
Definite NTM disease	6 (55%)	3 (14%)	0.033*
Possible NTM disease	4 (36%)	11 (50%)	0.712
Not NTM disease	1 (9%)	8 (36%)	0.212
Pulmonary symptoms (cough, dyspnea, hemoptysis)	8 [c] (73%)	11 (50%)	0.141
Constitutional symptoms (fever, weight loss, anorexia, etc.)	9 (82%)	15 (68%)	0.681
Median CD4 count (range)	131 [a] (14-502)	263 (1-617)	0.434 [d]
Abnormal CXR	6 (55%)	6 [b] (27%)	0.250
Treated as NTM	4 (36%)	4 (18%)	0.391
Died	2 (18%)	0	0.104

Column percentages

Abbreviations: MAC, Mycobacterium avium complex; NTM, nontuberculous mycobacteria; PLHIV, people living with HIV, CXR, chest x-ray

[a] *only 10 patients with CD4*

[b] *one patient had missing x-ray results*

[c] *excluded was patient with extrapulmonary MAC*

[d] *Mann-Whitney u test was used*

**significant at p-value ≤ 0.05*

Identifying NTM to species level is important, especially among PLHIV as it does not only aid in determining the clinical significance of the NTM isolate, but more importantly, proper treatment can be dependent in the specific NTM species identified. Isolation of *M. kansasii* or *M. avium* from the sputum of an HIV-positive patient will likely have a different diagnostic and therapeutic approach compared to the isolation of *M. gordonae* from a similarly nonsterile specimen [48]. Despite its importance, there is still paucity of the necessary facilities and resources to speciate NTM in the Philippines. The traditional biochemical and phenotypic process in NTM identification are not only tedious and time-consuming because of the slow growth of the mycobacteria but the results can also be inaccurate as it also depends in meeting culture conditions for a particular NTM specie. Most laboratories in our country use MPT64 testing to rapidly differentiate MTB and NTM but its clinical utility is more for the rapid identification of MTB and not to identify NTM. However, a negative MPT64 result does not rule out an MTB infection or co-existence of MTB and NTM. In our study, there were 6 cultures initially identified as NTM using MPT64 but were later determined to be *M. tuberculosis* after speciation using REBA Myco-ID test. Possible explanations for this are the insufficient production of MPT64 due to contamination of the specimens, mutation in the *mpt64* gene and possibly non-viable isolates [49-50]. Our study used the PCR-based REBA Myco-ID (M and D, Republic of Korea), a relatively new technology to speciate the NTM. It does not only has a rapid turnaround time which can avoid delays in management; but is also highly sensitive and specific. Reported in other studies are sensitivities of 100% and 97.5% in detecting MTB and NTM, respectively [51] and 98% concordance with culture for identifying and differentiating between MTB and NTM [30]. Another advantage of the REBA Myco-ID is the identification of possible mixed NTM species in the specimens, as cultures, real-time PCR and PCR- restriction fragment length polymorphism analysis (PRA) cannot identify mixed infections [30,51]. The REBA Myco-ID also has improved sensitivity due to the reduced size of PCR products [29].

General recommendations for *in vitro* susceptibility testing of NTM isolates are still limited. It is also controversial because other than macrolides, susceptibility patterns of anti-TB drugs like rifampicin and ethambutol for MAC are not reliable and lack correlation with clinical response [8,46]. For research purposes, we tested all NTM species for DST, regardless of clinical relevance; although it is noteworthy that not all species of NTM requires drug susceptibility testing in clinical practice [8,31]. The ATS/IDSA NTM guideline and Clinical Laboratory Standards Institute (CLSI) did not recommend a single method of DST for all NTM species. Broth-based methods like microdilution and macrodilution are recommended for MAC isolates, while modified proportion method can be used for *M. kansasii* and microdilution is generally recommended for rapidly-growing mycobacteria *like M. fortuitum* [31]. In our study, due to unavailability of broth-based DST methods in our laboratory and our limited funds, we performed proportion method for slowly-growing mycobacteria and Etest

for rapidly-growing mycobacteria. Etest has been evaluated as a potential alternative to broth microdilution for susceptibility testing of RGM; however, this test has not yet been standardized to provide results which can be correlated consistently with those obtained by microdilution [52]. Our study showed that most NTM species are not susceptible to standard TB drugs, thus the practice in our country of initially and presumptively treating symptomatic patients with AFB-smear positive sputum as TB can have negative serious outcomes especially among high-risk PLHIV who would turned out to have NTM disease. This diagnostic challenge is now lessen with the incorporation of GeneXpert MTB/RIF in the TB diagnostic algorithm.

A caveat is that the relationship of *in vitro* susceptibility of many NTM species poorly correlates with its clinical response. In fact, ATS and CLSI do not recommend reporting first-line TB drugs for isolates with MAC [8,31]. Clinicians should remember that using agents based from the *in vitro* DST may not eliminate some NTM disease in patients. Our resistance profile is comparable to the study done by Lan where PLHIV with MAC have more than 80% resistance to first-line TB medications [22].

In the setting of HIV, determining the actual clinical significance of an NTM isolate is a huge challenge for clinicians as some NTM species generally regarded as nonpathogenic or less pathogenic in the general population can be clinically relevant in PLHIV [8,53,54]. An NTM isolated from a nonsterile specimen of an HIV-positive patient can be a true infection, a colonization, or a contamination. The 2007 ATS/IDSA statement for NTM diagnosis and treatment has been specific and strict with its criteria in diagnosing NTM pulmonary disease. In our present study, if we rigorously adhere to this guideline, none of our subjects would have satisfied the strict criteria for definite NTM pulmonary disease mainly because these individuals submitted only single specimens for cultures and the guideline required multiple positive specimens for diagnosis. Presently, there is no guideline for the interpretation of NTM isolates from single sputum specimen from PLHIV. Several studies have reported low percentage of PLHIV fulfilling the strict case definitions of pulmonary NTM disease [9,48]. Although universally accepted, most data used in this guideline came from US studies, thus, its utility in resource-limited and TB- endemic countries like the Philippines remains to be determined. These findings may suggest that diagnosing NTM infection or disease solely on ATS/ IDSA case definitions may not be sufficient or reliable for a specific population like PLHIV.

Conclusion

Diagnosing clinically significant NTM disease among PLHIV may require correlating all the clinical, radiologic and microbiologic findings in the patient. PLHIV with NTM disease may present with non-specific symptoms of fever, chronic cough and weight loss, a low CD4 count and an abnormal chest x-ray. The most common abnormality in the chest radiographs of PLHIV with isolated NTM in their respiratory specimens were non-specific infiltrates followed by nodularities. Most patients with significant NTM disease were initially and presumptively treated for TB before subsequently managed for NTM. The most common NTM species isolated among PLHIV in this study were the less pathogenic *M. fortuitum* followed by *Mycobacterium avium* complex. Drug susceptibility studies showed MAC was 100% susceptible to clarithromycin while *M. fortuitum* was 100% sensitive to amikacin and ciprofloxacin. However, most NTM species were resistant to standard TB medications.

Limitations of The Study

First, this was a retrospective chart review involving a small number of patients. There were missing data from the charts and missing medical records of patients which precluded to a more accurate description and analysis. There were also many patients with unknown outcomes due to numerous PLHIV who were lost to follow-up. Since there was only a small number of patients, some of the significance of NTM may be overlooked. Patients were not followed prospectively to further determine the significance of NTM. Second, there was the possibility of contamination in several specimens as evidenced by the isolation of known NTM contaminants such as *M. gordonae, M. terrae* and *M. mucogenicum*. This contamination may be related to the methods of specimen collection, water supply in the laboratory or contamination of laboratory equipment. In our study, method of specimen collection from PLHIV were unknown or were not standardized. Third, chest x-ray readings were also not standardized as each chest x-ray plate was read by a different radiologist.

Recommendations

1) A large-scale and prospective study is recommended to further elucidate the clinical significance of nontuberculous mycobacteria among PLHIV. Patients, especially those categorized in the possible NTM and/or not NTM disease should be prospectively evaluated clinically and with aid of serial microbiologic and radiologic studies including CT scan to determine if there will be progression to true NTM disease during their course.
2) Since TB is more common in our country, another study that will directly compare the clinical, microbiologic and radiologic characteristics of patients with definite NTM disease to those with MTB, especially among PLHIV will help clinicians to discriminate the 2 mycobacterial diseases and to arrive with the proper diagnosis to avoid giving unnecessary treatment. MTB and NTM co-infections should also be characterized.
3) In the Philippines where the National TB program is anchored diagnostically in AFB smears, clinicians should be cautious and prudent in presumptively and empirically treating patients who are smear-positive as tuberculosis since smear positivity may also mean NTM infection. Additional diagnostic tests like cultures and GenExpert MTB/RIF, if available will help to prevent misdiagnosing these smear-positive patients.
4) Clinicians should pursue speciation of NTM isolates whenever available, especially if with high index of suspicion for NTM disease. Knowing the specific NTM species will help to determine the clinical significance of the isolate and to provide

the appropriate treatment regimen since NTM diseases are not manage similarly.

5) Applying the ATS/IDSA criteria in diagnosing NTM disease should be taken with caveat and prudence since this guideline still needs validation for use among HIV-infected patients and in countries where TB is endemic and resources are limited like the Philippines.
6) Patients should always be instructed about correct sputum collection for mycobacterial smears and cultures because improperly collected specimens (e.g. not rinsing the mouth with distilled water prior to collection) could lead to contamination and false-positive results.
7) Since NTM disease is more common in patients with low CD4, early screening and diagnosis of individuals who are high-risk for HIV can help prevent this opportunistic infection. Early anti-retroviral drugs among PLHIV without contraindications may also impede significant NTM disease.
8) With the rising cases of HIV/AIDS in our country, the government should provide necessary and free diagnostic facilities to aid in NTM diagnosis.

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References

1. Trinh QM, Nguyen HL, Nguyen VN, et al. Tuberculosis and HIV co-infection - focus on the Asia-Pacific region. Int J Infect Dis. 2015; 32: 170-178.
1. www.who.int/tb/challenges/hiv/tbhiv_factsheet_2013.pdf
2. Salvana EMT, Leyritana K, Alejandria MM, et al. Opportunistic infections in Filipino HIV patients. IDSA ID Week Poster Abstract Session: HIV-Associated Infections and Malignancies. 2012.
3. Salas L, Salvana EMT. Improving Opportunistic infection rates in Filipino HIV patients with increased access to antiretroviral therapy. Open Forum Infect Dis. 2016; 3.
4. Sterling TR, Pham PA, Chaisson RE. HIV-Infection related tuberculosis: clinical manifestations and treatment. Clin Infect Dis. 2010; 50: S223-S230.
5. Swaminathan S, Padmapriyadarsini C, Narendran G. HIV-associated tuberculosis: clinical update. Clin Infect Dis. 2010; 50: 1377-1386.
6. Chanda-Kapata P, Kapata N, Klinkenberg E, et al. Non-tuberculous mycobacteria (NTM) in Zambia: prevalence, clinical, radiological and microbiological characteristics. BMC Infect Dis. 2015; 15: 500.
7. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007; 175: 367-416.
8. McCarthy KD, Cain KP, Winthrop KL, et al. Nontuberculous mycobacterial disease in patients with HIV in Southeast Asia. Am J Respir Crit Care Med. 2012; 185: 981-988.
9. van Ingen J. Diagnosis of nontuberculous mycobacterial infections. Sem Respir Crit Care Med. 2013; 34: 103-109.
10. Wassilew N, Hoffmann H, Andrejak C, et al. Pulmonary disease caused by nontuberculous mycobacteria. Respiration. 2016; 91: 386-402.
11. www.bacterio.net
12. Henkle E, Winthrop K. Nontuberculous mycobacteria infections in immunosuppressed hosts. Clin Chest Med. 2015; 36: 91-99.
13. Prevots DR, Marras TK. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. Clin Chest Med. 2014; 36: 13-34.
14. Kendall BA, Winthrop KL. Update on the epidemiology of pulmonary nontuberculous mycobacterial Infections. Sem Respir Crit Care Med. 2013; 34: 87-94.
15. Thomson RM. Changing epidemiology of pulmonary nontuberculous mycobacteria infections. Emerg Infect Dis. 2010; 16: 1576-1583.
16. Gopinath K, Singh S. Nontuberculous mycobacteria in TB-endemic countries: are we neglecting the danger?. PLoS Negl Trop Dis. 2010; 4: e615.
17. Nyamogoba HDN, Mbuthia G, Mining S, et al. HIV co-infection with tuberculous and nontuberculous mycobacteria in Western Kenya: challenges in the diagnosis and management. Afr J Health Sci. 2012; 12: 305-311.
18. Kwon YS, Koh W. Diagnosis of pulmonary tuberculosis and nontuberculous mycobacterial lung disease in Korea. Tuberc Respir Dis. 2014; 77: 1-5.
19. Nyamogoba HDN, Mbuthia G, Kikuvi G, et al. A high tuberculosis and human immunodeficiency virus co-infection rate and clinical significance of nontuberculous mycobacteria in Western Kenya. Afr J Health Sci. 2012; 21: 147-154.
20. Bjerrum S, Oliver-Commey J, Kenu E, et al. Tuberculosis and nontuberculous mycobacteria among HIV-infected individuals in Ghana. Trop Med Int Health. 2016; 21: 1181-1190.
21. Lan R, Yang C, Lan L, et al. Mycobacterium tuberculosis and nontuberculous mycobacteria isolates from HIV-infected patients in Guangxi, China. Int J Tuberc Lung Dis. 2011; 15: 1669-1674.
22. Murcia-Aranguren MI, Gómez-Marin JE, Alvarado FS, et al. Frequency of tuberculous and nontuberculous mycobacteria in HIV-infected patients from Bogota, Colombia. BMC Infect Dis. 2001; 1: 21.
23. Archana C, Sarita K, Mangala G, et al. Study of

mycobacteriological profile among HIV-seropositive patients. Int J Healthcare Biomed Res. 2014; 2: 73-79.

24. Montoya JC, Santiago E, Manalo M, et al. Clinical, radiologic and microbiologic features of mycobacterial infection in patients with HIV. Phil J Microbiol Infect Dis. 1998; 27: 97-101.
25. Siapno RPV, King Kay CBO, Visperas JCG. The incidence of mycobacterium other than tuberculosis isolated in specimens suspected of tuberculosis in a tertiary hospital in the Philippines: a retrospective study. Phil J Int Med. 2016; 54: 1-4.
26. https://www.pgh.gov.ph/
27. Destura RV, Berba RP, Mendoza MT, et al. Profile of HIV/AIDS patients at the Philippine General Hospital: revisiting 9 years of clinical experience. Phil J Microbiol Infect Dis. 2003; 32: 11-21.
28. https://sfamjournals.onlinelibrary.wiley.com/doi/pdfdirect/10.1111/jam.12670
29. Wang HY, Bang H, Kim S, et al. Identification of Mycobacterium species in direct respiratory specimens using reverse blot hybridization assay. Int J Tuberc Lung Dis. 2014; 18: 1114-1120.
30. www.clsi.org
31. Biehle JR, Cavalieri SJ, Saubolle MA, et al. Evaluation of Etest for susceptibility testing of rapidly-growing mycobacteria. J Clin Microbiol. 1995; 33: 1760-1764.
32. Koh W-J, Yu C-M, Suh GY, et al. Pulmonary TB and NTM lung disease: comparison of characteristics in patients with AFB smear-positive sputum. Int J Tuberc Lung Dis. 2006; 10: 1001-1007.
33. Miguez-Burbano MJ, Flores M, Ashkin D, et al. Nontuberculous mycobacteria disease as a cause of hospitalization in HIV-infected subjects. Int J Infect Dis. 2006; 10: 47-55.
34. Alvarez-Uria G, Falco V, Martin-Casabona N, et al. Nontuberculous mycobacteria in the sputum of HIV-infected patients: infection or colonization?. Int J of STD & AIDS. 2009; 20: 193-195.
35. Kim YK, Hahn S, Uh Y, et al. Comparable characteristics of tuberculous and nontuberculous mycobacterial cavitary lung diseases. Int J Tuberc Lung Dis. 2014; 18: 725-729.
36. Saritsiri S, Udomsantisook N, Suankratay C. Nontuberculous mycobacterial infections in King Chulalongkorn Memorial Hospital. J Med Assoc Thai. 2006; 89: 2035-2046.
37. Thanachartwet V, Desakorn V, Duangrithi D, et al. Comparison of clinical and laboratory findings between those with pulmonary tuberculosis and those with nontuberculous mycobacterial lung disease. Southeast Asian J Trop Med Public Health. 2014; 45: 85-94.
38. http://www.ship.ph/wp-content/uploads/2018/11/CPGs-2016-tuberculosis.pdf
39. Miller WT Jr. Spectrum of pulmonary nontuberculous mycobacterial Infection. Radiology. 1994; 191: 343-350.
40. Pintado V, Gómez-Mampaso E, Martin-Davila P, et al. Mycobacterium kansasii infection in patients infected with the human immunodeficiency virus. Eur J Clin Microbiol Infect Dis. 1999; 18: 582-586.
41. Hoefsloot W, van Ingen J, Andrejak C, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples. An NTM-NET study. Eur Respir J. 2013; 42: 1604-1613.
42. Park S, Suh GY, Chung MP, et al. Clinical significance of Mycobacterium fortuitum isolated from respiratory specimens. Respir Med. 2008; 102: 437-442.
43. Smith MB, Schnadig VJ, Boyars MC, et al. Clinical and pathologic features of Mycobacterium fortuitum infections: an emerging pathogen in patients With AIDS. Am J Clin Patho. 2001; 116: 225-232.
44. Shafer RW, Sierra MF. Mycobacterium xenopi, Mycobacterium fortuitum, Mycobacterium kansasii, and other nontuberculous mycobacteria in an area of endemicity for AIDS. Clin Infect Dis. 1992; 15: 161-162.
45. Corti M, Palmero D. Mycobacterium avium complex infection in HIV/AIDS patients. Expert Rev Anti-infect Ther. 2008; 6: 351-363.
46. Horsburgh CR Jr, Gettings J, Alexander LN, et al. Disseminated Mycobacterium avium-complex disease among patients infected with human immunodeficiency virus, 1985-2000. Clin Infect Dis. 2001; 33: 1938-1943.
47. Corbett EL, Blumberg L, Churchyard GJ, et al. Nontuberculous mycobacteria: defining disease in a prospective cohort of South African miners. Am J Respir Crit Care Med. 1999; 160: 15-21.
48. Ang CF, Cajucom MAM, Kim Y, et al. Evaluation of a rapid assay for identification of Mycobacterium tuberculosis grown in solid and liquid media. Int J Tuberc Lung Dis. 2011; 15: 1475-1477.
49. Martin A, Bombeeck D, Mulders W, et al. Evaluation of the TB Ag MPT64 rapid test for the identification of Mycobacterium tuberculosis complex. Int J Tuberc Lung Dis. 2011; 15: 703-705.
50. Wang HY, Kim H, Kim S, et al. Evaluation of PCR-reverse blot hybridization assay for the differentiation and identification of Mycobacterium species in liquid cultures. J Appl Microbiol. 2015; 188: 142-151.
51. Woods GL, Bergmann JS, Witebsky FG, et al. Multisite reproducibility of Etest for susceptibility testing of Mycobacterium abscessus, Mycobacterium chelonae, and Mycobacterium fortuitum. J Clin Microbiol. 2000; 38: 656-661.
52. Barber TW, Craven DE, Farber HW. Mycobacterium gordonae: a possible opportunistic respiratory tract pathogen in patients with advanced human immunodeficiency virus, type 1 infection. Chest. 1991; 100: 716-720.
53. Lessnau K-D, Milanese S, Talavera W. Mycobacterium gordonae: A treatable disease in HIV-positive patients. Chest. 1993; 104: 1779-1785.

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