Nontuberculous Mycobacterial Species and Mycobacterium Tuberculosis Complex Coinfection in Patients with Pulmonary Tuberculosis in Dr. Soetomo Hospital, Surabaya, Indonesia

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Objective/Background: The aim of this study was to analyze the detection of nontuberculous mycobacterial (NTM) species derived from sputum specimens of pulmonary tuberculosis (TB) suspects. Increasing prevalence and incidence of pulmonary infection by NTM species have widely been reported in several countries with geographical variation. Materials and Methods: Between January 2014 and September 2015, sputum specimens from chronic pulmonary TB suspect patients were analyzed. Laboratory examination of mycobacteria was conducted in the TB laboratory, Department of Clinical Microbiology, Dr. Soetomo Hospital, Surabaya. Detection and identification of mycobacteria were performed by the standard culture method using the BACTEC MGIT 960 system (BD) and Lowenstein–Jensen medium. Identification of positive Mycobacterium tuberculosis complex (MTBC) was based on positive acid-fast bacilli microscopic smear, positive niacin accumulation, and positive TB Ag MPT 64 test results (SD Bioline). If the growth of positive cultures and acid-fast bacilli microscopic smear was positive, but niacin accumulation and TB Ag MPT 64 (SD Bioline) results were negative, then the isolates were categorized as NTM species. MTBC isolates were also tested for their sensitivity toward first-line anti-TB drugs, using isoniazid, rifampin, ethambutol, and streptomycin. Results: From 2440 sputum specimens of pulmonary TB suspect patients, 459 isolates (18.81%) were detected as MTBC and 141 (5.78%) as NTM species. Conclusion: From the analyzed sputum specimens, 18.81% were detected as MTBC and 5.78% as NTM species. Each pulmonary TB suspect patient needed clinical settings to suspect causative agents of MTBC and/or NTM species; clinicians have to understand the local epidemiological data for the evaluation of causes of lung infection to determine appropriate therapy.

Keywords: Dr. Soetomo Hospital, Surabaya, Mycobacterium tuberculosis complex, nontuberculous mycobacterial, sputum

INTRODUCTION

Increasing prevalence, incidence, and mortality of pulmonary infection caused by nontuberculous mycobacterial (NTM) species have been reported in various countries. NTM species are mostly found in a human or an animal environment, in water (especially tap water) and in soil; more than 150 species had been detected (identified) to date. Infection by NTM species is more frequently detected in pulmonary than in extrapulmonary infections. NTM species in both extrapulmonary and pulmonary infection diseases can be detected in both immunocompromised and immunocompetent humans. NTM infection has also been found to be one of the causes of opportunistic infection diseases such as pulmonary infection, especially in immunocompromised patients such as human immunodeficiency virus (HIV)-infected patients, patients on immunosuppressant therapy, those with TB and diabetes, and other comorbid patients. Necrosis of pulmonary tissues with airflow disruption resulted in decreased lung immunity.
which also contributes as a risk factor of NTM infections, such as chronic lung diseases, pulmonary cystic fibrosis, bronchiectasis, and cavity. NTM infection can occur both in immunocompromised and immunocompetent humans with structural abnormalities in lung tissue. An increase of coexistence detection NTM species of NTM in patients with pulmonary TB with anti-TB therapy can occur before, during, or after therapy program completed. Epidemiological data of NTM infection have been found to vary depending on geographical areas; therefore, it is necessary to conduct epidemiological surveillance to provide local epidemiological data. The evidence based are used as a basis for determining management of patients or empiric therapy procedures for pulmonary NTM infection suspect patients according to the standard guidelines of ATS 2007. An understanding of the local epidemiological data on NTM infection also functions as a basis for determining whether anti-NTM therapy is needed or not, depending on whether the NTM species were detected as causative pathogens, colonizers, or specimen contaminants.

Local prevalence epidemiological data of NTM species are often found to be correlated significantly with clinical setting, and so these can be considered in chronic pulmonary disease management. In addition, virulence detection of NTM species such as Mycobacterium avium complex (MAC) including Mycobacterium intracellulare, Mycobacterium abscessus, and Mycobacterium kansasii also correlates significantly with the clinical setting. In contrast, less virulent NTM species, such as Mycobacterium fortuitum and Mycobacterium chelonae, can be considered as causative agents of pulmonary infections, especially in an immunosuppressive condition. The findings of NTM species in infectious pulmonary can be multiple or only one type of species, virulent species or less virulence, opportunistic or not. Differences in sensitivity of antibiotic drugs toward various NTM species also need to be taken into consideration, but because of difficulty in distinguishing clinical manifestations, it is necessary to determine NTM species based on the laboratory determination of mycobacteria down to the species level. Diagnosis of pulmonary infection disease by NTM species, recommended by Griffith et al. and the Japanese Society for TB in 2008, was based on clinical manifestations, such as symptoms and signs, radiological pictures of high-resolution computed tomography (HRCT) scan of the lungs, and positive detection of NTM species.

As in the case of pulmonary TB, laboratory examination of mycobacteria is also recommended for suspect NTM pulmonary infection diseases before antibiotic therapy, during therapy at 1–2 months after starting treatment, and after therapy completion. Mycobacterial laboratory diagnosis for determining diagnosis at the start of therapy is also important for monitoring the results of therapy. Thus, clinical and specimen diagnoses are accurate to determine the validity of diagnosis and choice of therapy.

Effective communication is necessary between clinical lung disease specialists and clinical microbiology specialists to determine the causative agents and clinical significance precisely as a basis for adequate patient management. The aim of this research was to analyze the detection of NTM species from sputum specimens of pulmonary TB suspect patients.

**MATERIALS AND METHODS**

This research was conducted from January 2014 to September 2015. Prior to and during treatment with first-line anti-TB drugs, almost all patients were identified to have chronic pulmonary TB. Sputum specimens from 2440 patients of pulmonary TB suspects were analyzed. Specimens consisted of 2212 spontaneous and 228 BAL sputum samples. All samples were received from in- and out-patients of Dr. Soetomo Hospital Surabaya, a tertiary medical center in Indonesia. This study was approved by the Ethics Committee in Health Research of Dr. Soetomo General Hospital, Surabaya, in Indonesia (no. 124/Panke. KKE/II/2014). The pulmonologist in Dr. Soetomo Hospital was responsible for obtaining informed consent from patients with TB.

Spontaneous sputum and BAL specimens were examined in the TB Laboratory, Department of Clinical Microbiology, Dr. Soetomo Hospital, Surabaya. The standard culture method including BACTEC MGIT 960 system (BD) liquid medium and that using Lowenstein–Jensen solid medium were used for the detection and identification of mycobacteria. After a positive growth of mycobacteria, identification of M. tuberculosis complex (MTBC) was based on acid-fast bacilli positive smear using Ziehl–Neelsen method, positive accumulation niacin test (BD), and positive TB Ag MPT64 test (SD Bioline). MTBC isolates were then tested for susceptibility to the first-line anti-TB drugs, that is, isoniazid, rifampin, ethambutol, and streptomycin. If both culture growth and acid-fast bacilli smear results were positive, but niacin test accumulation and TB Ag MPT 64 (SD Bioline) test results were negative, isolates were considered NTM species.

**RESULTS**

Laboratory tests of mycobacteria for the detection–identification of MTBC and NTM species from 2212 spontaneous sputum specimens were carried out during January 2014–September 2015. BAL specimens were obtained from 228 patients, and 58 of these specimens examined were from both right and left lungs. Detection and identification results were positive for two BAL specimens derived from one patient with the same type of mycobacteria isolated, either MTBC or NTM species, while in specimens from 170 patients with a single BAL sputum sample, only one type of mycobacterium was found.

Spontaneous sputum of each patient was examined once. In both types of specimens, spontaneous sputum and BAL, only one type of MTBC and/or NTM species could be detected. A number of 2440 specimens with 2212 spontaneous sputum of lower respiratory specimens and 228 BAL indicated...
examination of microbiology laboratory with clinical diagnosis of suspected pulmonary TB, coinfection of MTBC with chronic lung disease, such as bronchiectasis, pleural effusion, malignancy, comorbid HIV, coagulase infection, and others.

Only one sputum specimen from each patient was examined using culture method identification of MTBC and NTM species. MTBC isolates were tested to determine their sensitivity to the first-line anti-TB drugs. BAL sputum of 58 patients was taken from both lungs; single BAL was examined from 170 patients.

During examination of spontaneous sputum, out of 2212 identification-detected patients, 411 isolates (18.58%) were MTBC positive and 117 (5.29%) were NTM positive; among 228 BAL specimens examined, 48 isolates (21.05%) were detected as MTBC positive and 24 (10.53%) as NTM positive. From a total of 2440 specimens, 459 (18.81%) isolates were detected as MTBC positive and 141 (5.78%) as NTM positive [Table 1]. The first-line anti-TB sensitivity tests also detected 23 (5.01%) MDR-TB isolates from 459 MTBC-positive specimens of suspected TB patients.

**Discussion**

In this study, 411 MTBC (18.58%) and 117 NTM (5.29%) isolates were detected from 2212 spontaneous sputum specimens, while 48 (21.05%) MTBC and 24 (10.53%) NTM isolates were identified from 228 BAL specimens; all specimens were derived from 2440 comorbid chronic TB suspect patients in the hospital. Isolation of NTM species from clinical specimens, mainly derived from the respiratory system, was commonly reported in TB-endemic countries, in addition to MTBC or coinfection.\(^2\)

Most studies reported a great diversity of NTM species distribution according to geographical areas; thus, epidemiologic surveillance activities should be conducted in every geographical area. In a previous study conducted in Taiwan during 2000–2012, among 13,652 samples examined from TB suspect patients with mycobacteria-positive respiratory culture, 7774 (56.9%) were found to be NTM positive. From a total of 2440 specimens, 459 (18.81%) isolates were detected as MTBC positive and 24 (10.53%) as NTM positive [Table 1]. The first-line anti-TB sensitivity tests also detected 23 (5.01%) MDR-TB isolates from 459 MTBC-positive specimens of suspected TB patients.

The prevalence of NTM diseases and colonization increased particularly among older patients, with half of these patients being ≥65 years old. In 3317 patients who had NTM infection, the most prevalent species were *M. avium*–*M. intracellulare* complex (MAC) (1377; 41.5%) and *M. abscessus* (710; 21.4%). The prevalence of NTM pulmonary infection and colonization was found to be significantly increased during the study period.\(^2\) Pulmonary infection cases most commonly occurred due to *M. avium, M. kansasii, and M. abscessus*.\(^3\)

Shao et al.\(^1\) reported that among 1779 positive clinical isolates from patient sputum in the eastern region of China, 60 (3.37%) were detected as NTM positive, with *M. intracellulare* (68.33%) being found to be the predominant species, followed by *M. abscessus–Mycobacterium immunogenum* (13.33%), *Mycobacterium* spp. (10.00%), *M. kansasii* (6.67%), and *Mycobacterium peregrinum–Mycobacterium alvei–Mycobacterium septicum* (1.67%). Misdiagnosis of NTM disease as MDR-TB frequently occurred in clinical settings because of similar clinical manifestations, especially in high-MTBC-burden countries. Different NTM species exhibited various pathogenicity and antibiotic susceptibility patterns; NTM species were frequently found to be resistant toward the first-line anti-TB drugs. Shahrai et al.\(^1\) revealed that out of 117 strains isolated from MDR-TB suspect patients, 30% were identified as NTM species using conventional culture and molecular method due to a possibility of NTM misdiagnosis with MDR-TB, as indicated by a lack of response toward anti-TB treatment.

D‘Antonio et al.\(^1\) also reported that in respiratory wards of S. Camillo Forlanini Hospital, Italy, during January 2011–December 2013, clearly there was a link between the significant NTM contamination of hospital water pipelines and an increase of NTM patient airway colonization. Moreover, sputum analysis showed a significant increase in NTM isolation rate in patients referred to the respiratory wards over time. In 2011, only 18% of 190 patients with sputum samples positive for acid-fast bacilli were positive for NTM, which increased in the second trimester of 2013, with 53.33% of sixty patients being positive for NTM. MAC was the most frequent NTM subtype with the frequency ranging from 61.5% to 87%, followed by *Mycobacterium gordonae, Mycobacterium xenopi*, and *M. chelonae M. fortuitum, Mycobacterium simiae, M. kansasi*, and *M. abscessus* were also found in small percentages.

van Halsema et al.\(^1\) reported that in a study in South Africa during July 2006–December 2007, 2496 individuals were recruited and their sputum samples were collected. A total of 720 specimens (28.8%) yielded mycobacterial growth; 58.5% grew *M. tuberculosis* and 41.5% grew NTM species. Among this TB case, revealed that 74/232 (31.9%) were infected by HIV. HIV prevalence was 12/15 (80.0%) for those with MAC, 14/22 (63.6%) for those with *M. kansasii*, and 11/13 (84.6%) for those with *M. gordonae*.

Studies conducted at the National TB and Leprosy Training Center, Zaria, Nigeria, from August 2010 to July 2011, and

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**Table 1: Pattern of mycobacterial isolates from spontaneous sputum and bronchoalveolar lavage specimens of suspected pulmonary tuberculosis patients at Clinical Microbiology Laboratory, Dr. Soetomo Hospital Surabaya, January 2014 to September 2015**

| Specimen type   | Number of specimens | MTBC positive (%) | NTM positive (%) |
|-----------------|---------------------|-------------------|------------------|
| Spontaneous sputum | 2212                | 411 (18.58)       | 117 (5.29)       |
| BAL             | 228                 | 48 (21.05)        | 24 (10.53)       |
| Total           | 2440                | 459 (18.81)       | 141 (5.78)       |

MTBC: *Mycobacterium tuberculosis* complex, NTM: Nontuberculous mycobacterial, TB: Tuberculosis, BAL: Bronchoalveolar lavage
at the Barau Dikko Hospital, Kaduna City, from December 2010 to July 2011, also reported NTM infection cases to explain its prevalence. Sputum samples from 1603 consecutive new cases with presumptive diagnosis of TB were collected from August 2010 to July 2011. Out of the 1603 patients screened, 444 (28%) culture-positive cases of pulmonary TB were identified; 375 (85%) cases were detected to be caused by MTBC strain (354 cases of M. tuberculosis, 20 cases of Mycobacterium africanum, and 1 case of Mycobacterium bovis), and 69 (15%) cases by NTM infection. An understanding of the local epidemiologic data is crucial for clinicians to evaluate positive culture examination results, whether it was MTBC or NTM species; any pulmonary TB suspect required a clinical setting, in consideration of pulmonary TB suspect and lung infection by NTM species. In the past decade, a high incidence of NTM infection among the elderly population globally had resulted in significantly high mortality and morbidity rates. Bronchiectasis, chronic lung diseases, pulmonary fibrosis, and decreased lung immunity were some of the risk factors of pulmonary NTM diseases. NTM pulmonary diseases formed a broad clinical spectrum, ranging from asymptomatic infection to fatal disseminated disease. Many patients with NTM pulmonary diseases had other underlying diseases. NTM disease symptoms were indistinguishable from chronic pulmonary structural diseases. Therefore, diagnosis of NTM infection using the conventional method might be misleading and needed the molecular method as a complementary test to detect NTM species to improve patient care.

Suspected pulmonary infection by NTM species was based on clinical criteria including clinical symptoms and patient complaints, radiological pictures of HRCT scan of the lungs, along with the diagnosis of pulmonary infection by NTM species based on NTM species detection in patient respiratory specimens according to the method of Griffith et al. In the current study, identification of NTM isolates at species level was yet to be conducted due to facility limitation. Determining NTM isolates down to species level was crucial for implementing adequate therapy. Ten NTM species, including virulent NTM species MAC, M. abscessus, and M. kansasii, were found as causative pathogens of lung infection in relation to the importance of species determination, the three species had different sensitivity toward various antimicrobials.

However, species determination could be used as interpretation basis of whether mycobacteria isolated were causative pathogens NTM species of lung infection, a colonization, or a contamination in relation to various NTM species commonly found in human or animal environment. Among the pulmonary TB suspect patients examined in the current study, 5.01% were found to have MDR-TB. This finding was important for the treatment of patients suspected to have pulmonary TB. Accurate specimens supported precise diagnosis. Respiratory specimens derived from NTM pulmonary infection suspect patients were taken according to the ATS guideline standard, 3 repeating sputum collected mainly on morning and early morning sputum specimens taken on different days.

Accurate sputum specimen collection and appropriate clinical suspect could determine laboratory examination methods for mycobacteria, including decontamination and concentration sputum with standard method, isolation using liquid and solid medium culture, examination method that was conducted at least twice in selective media, incubation method, and detection–identification process, to increase sensitivity and specificity. A previous study reported that NTM species were also found from pulmonary TB patients during anti-TB treatment, but whether it was colonization, transient infection, or contamination of the specimen was yet to be determined. Coinfection of TB and NTM species in lung infection disease was rarely reported; however, it should also be considered as a coinfection in the findings virulence NTM species such as M. abscessus.

Infection of NTM species was also reported to be related to Aspergillus lung infection, including aspergilloma and chronic necrotizing pulmonary aspergillosis. Diagnosis of NTM infection was established based on the findings of NTM species from replicated respiratory specimens, with positive NTM detection on more than two sputum specimens taken on different days and identification of virulent NTM species as causative pathogens according to clinical manifestations and radiological pictures of pulmonary HRCT scan. Therefore, providers should consider NTM infection in every patient suspected to have chronic pulmonary TB.

Identification of MTBC or NTM species is based on growth acceleration, colony morphology (i.e., characteristics and pigment), biochemical tests, antigen detection serological tests, and polymerase chain reaction DNA hybridization probe biomolecular tests. NTM species are identified by polymerase chain reaction restriction fragment length polymorphism based on region targets of 16S-23S rRNA, 16S rRNA, rpoB, or hsp 65 DNA genes, and other region targets. Other studies also reported that multiplex polymerase chain reaction was considered to be the most accurate and efficient diagnostic tool for the detection of NTM species and MTBC.

This observation specifically conducted to detect NTM in TB chronic cases along with lung tissue lesion based on chest X-ray radiological picture that stated all patients in severe disease category, all were inpatients in Dr. Soetomo Hospital, Surabaya, Indonesia. From the data, there is a need of further research to compare between active and nonactive diseases to elucidate the mechanism of coinfection and analyze the risk factors. The other important significance of further strategic research may be evidence-based identification of TB problems to determine strategic management in health-care system in Indonesia.
CONCLUSION

Examination of TB sputum specimens of 2440 patients with suspected pulmonary TB collected from January 2014 to September 2015 at the TB Laboratory, Department of Clinical Microbiology, Dr. Soetomo Hospital Surabaya, resulted in 459 (18.81%) MTBC, 141 (5.78%) NTM, and 5.01% MDR-TB isolates.

Every pulmonary TB suspect patient required clinical settings for suspect causative agents of MTBC or NTM species; clinicians should have an understanding of the local epidemiological data as an evaluation basis of lung infections, based on the ATS/IDS A NTM guidelines, TB guidelines, and recommendations of the World Health Organization and Indonesian Ministry of Health.

Accurate specimens should be obtained for representing respiratory samples necessary for diagnostic precision of infection-causing mycobacteria. Diagnosis of MTBC and NTM infection should be based on clinical symptoms, patient complaints, and radiological pictures of pulmonary HRCT scan, and then confirmed by the detection of MTBC or NTM species.

Pulmonary TB suspect or lungs comorbid diseases should be handled mainly on the findings of virulent NTM species such as MAC, M. abscessus, and M. kansasii. Less virulent NTM species possibly resulted from colonization or transient infection. Effective communication is needed between lung clinicians and clinical microbiology specialists to determine whether detected NTM species were causative pathogens, colonizers, or specimen contaminants, for adequate patient treatment.

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Conflicts of interest
There are no conflicts of interest.

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