IDENTIFICATION OF NONTUBERCULOUS MYCOBACTERIA (NTM) SPECIES ISOLATED FROM THE SPUTUM, SKIN AND SOFT TISSUE OF PATIENTS IN JAKARTA, INDONESIA

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Abstract. Knowing the species of nontuberculous mycobacteria (NTM) causing disease in a patient population can guide empiric treatment. In this study we aimed to determine the species of NTM causing disease in a selected patient population in Indonesia in order to guide empiric treatment. We retrospectively reviewed the records of all patients treated at 5 hospitals in Jakarta, Indonesia, diagnosed with NTM infection during January 2018-December 2019. The inclusion criterion for study subjects was having a positive test for NTM and the exclusion criterion was having a positive culture for *Mycobacterium tuberculosis* complex. Study subjects were confirmed to have NTM by a MPT64 rapid test. The species of NTM were identified by DNA sequencing of *Mycobacterium* 16S rRNA. A total of 15 patients were determined to have NTM at the study institutions during the study period, 53% male. The average age of study subjects was 45 (range: 4-83) years. The NTM positive specimens were obtained from the sputum (*n* = 5), skin and soft tissue biopsies (*n* = 5), and lymphoid tissue biopsies (*n* = 5). Of the 15 study subjects, 8 (53%) had *M. abscessus*, 5 (33%) had *M. intracellulare*, 1 (7%) had *M. avium* and 1 (7%) had *M. cookii*. Of the 5 NTM isolates from the sputum, 3 isolates were *M. abscessus*, 1 isolate was *M. intracellulare* and 1 isolate was *M. Cookii*. Of the 5 NTM isolates from skin and soft biopsies, 3 isolates were *M. abscessus* and the other 2 isolates were *M. intracellulare*. Of the 5 NTM isolates from lymphoid tissue biopsies, 2 isolates were *M. abscessus*, 2 isolates were *M. intracellulare* and 1 isolate was *M. Avium*. In our study, the most common NTIM isolate was *M. abscessus*. Empiric treatment of NTM in the study population should include coverage of *M. abscessus*. Further studies with larger numbers of subjects from multiple institutions are needed to determine if these findings hold true for other parts of Indonesia.

Keywords: NTM, *Mycobacterium*, sputum, skin and soft, lymphoid

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INTRODUCTION

Nontuberculous mycobacteria (NTM) is an acid-fast bacilli found worldwide (Dodiuk-Gad *et al*, 2007). One-hundred sixty species of NTM have been reported; 50 of these have been reported to be pathogens in humans (Griffith *et al*, 2007). NTM can cause infections similar to *Mycobacteria tuberculosis*; treatment is species dependent (Baldwin *et al*, 2019). There are no previous studies identifying the various NTM species causing infection in humans in Indonesia. Treatment of NTM in Indonesia is empiric, possibly leading to incorrect management. Identification of pathogenic species is important to guide empiric treatment.

There are commercial kits to identify NTM species that are less expensive and easier to use than DNA sequencing, but some kits have been reported to have problems with inadequate sensitivities, specificities and species identification (Lee *et al*, 2009; Hoza *et al*, 2016; Mok *et al*, 2017). Three genes (the 16S–23S internal transcribed spacer (ITS), the 16S rRNA and the *rpo*B) are commonly used to identify NTM via DNA sequencing (Liu *et al*, 2016). Of these 3 genes, the 16S rRNA and *rpo*B have been reported to be superior to the ITS for NTM species identification (Davari *et al*, 2019).

A study comparing the 16S rRNA genome sequence with the entire genome sequence found the phylogenetic trees constructed from these two sequences had substantial concordance (Fedrizzi *et al*, 2017). It has been reported DNA sequencing using the 16S rRNA sequence could be used as a gold standard and confirmation test for NTM species identification (Srinivasan *et al*, 2015).

NTM species vary widely by region (Hoefsloot *et al*, 2013; Kham-Ngam *et al*, 2018; Lim *et al*, 2018). Two previous studies from Indonesia attempted to identify the prevalence of NTM infection but not the causative species (Mertaniasih *et al*, 2017; Saptawati *et al*, 2019). In this study, we aimed to identify the NTM species causing infection in patients attending any of 5 hospitals in Jakarta, Indonesia in order to guide empiric treatment of NTM cases at these institutions.

MATERIALS AND METHODS

Clinical specimens

We retrospectively reviewed the charts of patients diagnosed with and treated for NTM at any one of 5 study hospitals in Jakarta, Indonesia during January 2018-December 2019. Samples included sputum, skin and soft tissue biopsies, and lymphoid tissue biopsies. Each sputum sample was decontaminated with an equal volume of N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) solution and each biopsy sample was decontaminated with 2 ml of NALC-NaOH.

Mycobacterial culture and MPT64 rapid test

Five hundred microliters of the decontaminated mixture prepared above was put in BD BACTEC MGIT 960 liquid medium (BD Biosciences, Sparks Glencoe, MD) and incubated for 21 days in order to detect Mycobacterial growth. Positive specimens were examined with the SD Bioline TB Ag MPT64 rapid test (Standard Diagnostics Inc, Suwon City, Kyonggi Province, Republic of Korea) in order to differentiate between NTM and *Mycobacterium tuberculosis* complex (MTBC); a positive test indicating MTBC. All isolates negative for MTBC were further examined by amplification of *Mycobacterium* 16S rRNA fragments.

PCR 16S rRNA and DNA sequencing

To amplify the 16S rRNA fragments (543 base pairs), 1 ml BACTEC liquid media (BD Biosciences, Sparks Glencoe, MD) was centrifuged at 12,000 rpm for 5 minutes and the resulting pellet was subjected to a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) giving a final elution product of 60 µl. To prepare for polymerase chain reaction (PCR) this product was then mixed with 1x PCR Master Mix Solution (i-MAX II DNA Polymerase) (iNtRON Biotechnology Inc, Gyeonggi-do, Republic of Korea), 0.4 µM of each of two primers and 2 µl DNA template. The primers used for the PCR (Forward: 5′- ACG GTG GGT ACT AGG TGT GGG TTT C -3′ and reverse: 5′- TCT GCG ATT ACT AGC GAC TCC GAC TTC A-3′) have been reported previously (Huard *et al*, 2003). The thermal cycling was performed under the following conditions: 94°C for 4 minutes; 40 cycles at 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 60 seconds and 72°C for 5 minutes. The PCR product was then electrophoresed on 2% agarose gel and documented using the Gel Documentation System (Bio-Rad, Hercules, CA). The PCR product was purified and then sequenced using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) and a BigDye® Terminator Sequencing Standard Kit v3.1 (Applied Biosystems, Foster City, CA). The obtained 16S rRNA sequences were deposited in the GenBank (accession no.: MT613412-MT613426).

The study was approved by the Ethics Committee, Faculty of Medicine, Universitas Indonesia (KET-435/UN2.F1/ETIK/PPM.00.02/2019).

RESULTS

A total of 15 patients were determined to have NTM at the study institutions during the study period, 53% male. The average age of study subjects was 45 (range: 4-83) years. The 543-bp 16S rRNA fragments were successfully amplified for all the 15 isolates (Fig 1). The sequences were obtained by DNA sequencing and the identification of the NTM species was analyzed in GenBank (data not shown). The NTM positive specimens were obtained from the sputum (*n* = 5), skin and soft tissue biopsies (*n* = 5) and lymphoid tissue biopsies (*n* = 5) (Table 1). Of the 15 study subjects, 8 (53%) had *M. abscessus*, 5 (33%) had *M. intracellulare*, 1 (7%) had *M. avium*, and 1 (7%) had *M. cookii*. Of the 5 isolates from the sputum, 3 isolates were *M. abscessus* and the other 2 isolates were *M. intracellulare* and M. cooki. Of the 5 isolates from the skin and soft tissue biopsies, 3 isolates were *M. abscessus* and the other 2 isolates were *M. intracellulare*. Of the 5 NTM isolates from the lymphoid tissue biopsies, 2 isolates were *M. abscessus*, 2 isolates were *M. intracellulare* and 1 isolate was *M. avium*.

DISCUSSION

Mycobacterial species can be categorized into rapidly growing mycobacteria (RGM) and slow growing mycobacteria (SGM) (Kim *et al*, 2013). Both RGM and SGM species have been reported to cause disease in humans (Hashemi Shahraki *et al*, 2017; Forbes *et al*, 2018). In this study, 8 isolates were RGM (all *M. abscessus*) and 7 were SGM (*M. avium*, *M. intracellulare*, and

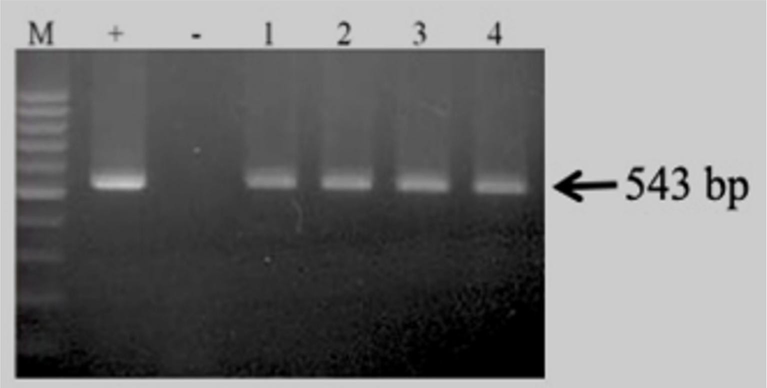

Fig 1 - 16S rRNA PCR results

Lane M: DNA ladder from 100 bp at the bottom to 1000 bp at the top; Lane +: positive control; Lane -: negative control; Lane 1: *Mycobacterium avium*; Lane 2: *M. abscessus*; Lane 3: *M. intracellulare*; Lane 4: *M. Cookie*

bp: base pairs; PCR: polymerase chain reaction

M. cookii) (Table 1). It is important to identify the NTM to the species level for effective adequate prognosis and therapy (Boyle *et al*, 2015; Leyer *et al*, 2017).

In Indonesia, there is limited data regarding NTM infections. In Surabaya and Surakarta, pulmonary NTM infections comprised 6% and 15% of sputum sample isolates obtained from patients suspected of having pulmonary TB but these isolates were not identified to the species level (Mertaniasih *et al*, 2017; Saptawati *et al*, 2019). In our study, the most common NTM species isolated from the sputum was *M. abscessus*, the same as 2 studies from Singapore (Lim *et al*, 2018; Schiff *et al*, 2019). In China, the most common NTM species isolated was *M. intracellulare* and in Tanzania it was M. gordonae. (Hoza *et al*, 2016; Liu *et al*, 2016).

In our study, the most common NTM species isolated from skin and soft tissue biopsies was *M. abscessus* and the most common NTM species isolated from lymphoid tissue biopsies were *M. abscessus* and *M. intracellulare*. The most common NTM species isolated from skin biopsies in Singapore (Lim *et al*, 2018), Thailand (Chairatchaneeboon *et al*, 2018) and Taiwan (Hsiao *et al*, 2011) was *M. abscessus*. The most common NTM species isolated from skin biopsies in the United States and Europe was *M. abscessus* (Wagner and Young, 2004) and in Israel was *M. marinum* (Dodiuk-Gad *et al*, 2007).

The usual regimen for treating *M. abscessus* is a macrolide, an aminoglycoside and cefoxitin, imipenem or tigecycline (Wu *et al*, 2018). Since *M. abscessus* was the most common NTM isolated in our study, these might be recommended as empiric treatment of NTM infections in the study population.

A strength of our study was the use

Table 1

The NTM species by type of sample

Species	Sample			Overall *n* (%)
	Sputum	Skin and soft biopsy	Lymphoid biopsy	
Mycobacterium abscessus	3	3	2	8 (53)
Mycobacterium intracellulare	1	2	2	5 (33)
Mycobacterium avium	0	0	1	1 (7)
Mycobacterium cookii	1	0	0	1 (7)
Total	5	5	5	15

NTM: nontuberculous mycobacteria

of molecular identification to identify the NTM species. A weakness of our study was the small number of clinical specimens and limited patient clinical data.

In summary, in our study we found the most common NTM species in the sputum and obtained from tissue biopsies was *M. abscessus*. We conclude empiric treatment of NTM infection in the study population should cover *M. abscessus*. Further studies are needed with greater numbers of patients and from multiple institutions to determine if these results hold true for other institutions.

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