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Conference Paper

Prevalence of Non-tuberculous Mycobacteria (NTM) in Surakarta, Indonesia: Higher Than Expected

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Abstract

The incidence and prevalence of infection due to non-tuberculous mycobacteria (NTM) species are increasing worldwide and rapidly becoming a major public health problem. Despite increasing knowledge about NTM, there are still many challenges in diagnostic tools and treatment for this infection. Indonesia is ranked number two for tuberculosis infections; however, there is a lack of data about NTM infection. The aim of this study was to analyze the prevalence of the detection of NTM from sputum specimens of pulmonary tuberculosis suspects in a lung hospital center in Surakarta, Central Java, Indonesia. This study was a retrospective study which analyzed the laboratory data obtained from a lung hospital (*Balai Besar Kesehatan Paru Masyarakat* / BBKPM), in Surakarta, Indonesia. Samples were collected from patients with tuberculosis and cases with a presumptive diagnosis of tuberculosis from January 2013 to December 2017. All sputum samples were stained for microscopic analysis and were also incubated for the detection of mycobacterial growth in Lowenstein Jensen (LJ) medium and in LJ medium with the addition of para-nitrobenzoic acid (LJ-PNB) to identify *Mycobacterium tuberculosis* (MTB) or NTM strain. Biochemistry test, Niacin test, was used to confirm NTM strain. For five year periods, there were 9,284 patients samples were cultured to detect MTB infection. For all the sputum samples, 1,974 samples (21.3%) were cultured positive; out of the positive samples 1,636 (85%) were MTB strains and 334 (15%) were NTM strains. The data revealed that NTM infection cases were more likely to have been diagnosed aged older than 35 years (72%). This study showed that there was a high number of NTM infection detected in patients with presumptive diagnosis of tuberculosis. Therefore, another approach should be conducted to provide effective therapy for infection due to NTM. Identification of NTM in species level will be important to provide empirical therapy for this infection.

1. Introduction

Non-tuberculous mycobacteria (NTM) are a group of Mycobacterium species other than *Mycobacterium tuberculosis* (MTB) and this group does not cause leprosy [1]. NTM is environment microbial that can be found in soil and aquatic environment [1]. It has been reported that NTM has been isolated from various equipment and environment such

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as drinking water pipelines, water tanks, hospital faucets and ice machines, diagnostic laboratories, bottled and municipal water, commercial and hospital ice, potting soil, house dust, water damaged building materials, showerheads, shower aerosols, hot-tub aerosols, livestock, and seawater [2]. NTM generally is harmless for most individual, however, this opportunistic pathogen can cause diseases in immunosuppressive individual or an individual who has pre-existing, underlying lung diseases [3].

There are more than 150 species of NTM that has been identified worldwide. The distribution of NTM species shows that this group is specific geographically. It is believed that NTM is generally acquired from the environment *via* ingestion, inhalation, and dermal contact, which results in lymphadenitis, pulmonary and disseminated infections, and skin and soft tissue infections [4]. NTM might vary in their pathogenicity but most commonly associated with pulmonary infections [5]. Clinical presentations of NTM include pulmonary infection, disseminated infection, skin disease, and lymphadenitis [3].

The incidence and prevalence of infection due to NTM species are increasing worldwide and diseases caused by NTM species rapidly becoming a major public health problem worldwide [3]. Studies showed that the prevalence of NTM has increased in high burden and non-high burden countries of tuberculosis. There is not clear what the cause of the increase of NTM infection worldwide. It has been suggested that the development and the availability of the methods for strain identification have increased the detection of NTM infection. The identification of NTM is very important since the failure to detect NTM infection has led to mistaken treatment for tuberculosis [6]. In addition, the treatment for NTM infection is species-specific [7].

Indonesia is a high burden country of tuberculosis and epidemiology of tuberculosis infection in Indonesia is well described; however, there is very limited information about the prevalence of NTM infection. In addition, the prevalence of multi-drug resistant tuberculosis in Indonesia is increasing [8]. Given the high prevalence of tuberculosis infection and the increase of MDR tuberculosis, there is a growing concern that NTM infections could be misdiagnosed as tuberculosis. Therefore, investigating the prevalence of NTM infection is crucial.

This present study aimed to show the prevalence of NTM which is detected from sputum samples of patients' presumptive tuberculosis infection in Surakarta, Indonesia.

2. Methods

2.1. Samples and study design

This study was a retrospective study which analyzed the laboratory data obtained from a lung hospital (*Balai Besar Kesehatan Paru Masyarakat* / BBKPM), Surakarta, Central Java Province, Indonesia. Samples were collected from patients with tuberculosis and patients with a presumptive diagnosis of tuberculosis from January 2013 to December 2017.

2.2. Detection of non-tuberculous mycobacteria (NTM)

All sputum samples from cases with tuberculosis and cases with presumptive diagnosis tuberculosis patients which were sent to BPKPM Lung Hospital, Surakarta were examined microscopically to detect the presence of acid-fast bacilli (AFB) using *Ziehl-Neelsen* (ZN) staining. The samples were also grown in Lowenstein Jensen (LJ) medium and LJ medium with the addition of para-nitrobenzoic acid (LJ-PNB) to differentiate between MTB and NTM. Samples which grew in both LJ and LJ-PNB media were NTM strains, while those which only grew in LJ medium were MTB strains. Biochemistry test, niacin accumulation test, was conducted to confirm the NTM strain.

2.3. Data analysis

The data were analyzed to see the prevalence of NTM in patients. The colonies grown in LJ medium with the addition of PNB were analyzed and cross-checked with the niacin test to confirm the presence of NTM strain in the patient's samples.

3. Results

A total of 9,284 sputum samples examined for detection of MTB infection from patients with tuberculosis and cases with a presumptive diagnosis of tuberculosis in the lung hospital (*Balai Besar Kesehatan Paru Masyarakat* / BBKPM) from January 2013 to December 2017. The percentage of the patient's sputum samples detected with NTM and MTB is shown in Table 1. Out of all the patient's samples that grew in LJ medium, 1,974 (25%) samples showed positive results. Of all these positive cultures, 334 (15%) grew in LJ-PNB medium and were identified as NTM strains. Colonies that only showed grow in LJ medium were 1,636 (85%) and were identified as MTB strains The biochemistry test, niacin, was conducted to confirm the NTM colony. The culture results were compared

to the smear examination of the sputum sample stained using ZN staining method. The comparison of sputum smear results and the bacterial growth in LJ-PNB is shown in Table 2. The data shows that only 38% of all samples showed positive for NTM were also positive for AFB in the sputum samples.

TABLE 1: The percentage of positive cultures of NTM. The NTM detection was conducted by culturing the sputum samples into LJ and LJ-PNB medium. Samples with NTM positive grew in both LJ and LJ-PNB medium.

Year	Total samples (n)	Negative cultures	Positive cultures		
			Total positive cultures	Culture positive for MTB	Culture positive for NTM
2013	1616	969 (60 %)	647 (40 %)	577 (89 %)	70 (11 %)
2014	1522	1034 (68 %)	488 (32 %)	408 (84 %)	80 (16 %)
2015	2209	1773 (80 %)	436 (20 %)	388 (89 %)	48 (11 %)
2016	2070	1587 (77 %)	483 (23 %)	403 (83 %)	80 (17 %)
2017	1867	1603 (86 %)	264 (14 %)	198 (75 %)	66 (25 %)
Total samples	9284	6966 (75 %)	2318 (25 %)	2179 (85 %)	334 (15 %)

TABLE 2: Comparison of a positive culture of Non-tuberculous mycobacteria (NTM) with the Acid Fast Bacilli (AFB) detection by microscopic examination (Ziehl-Neelsen staining).

Total culture positive for NTM (n=334)	Sputum samples AFB positive	Sputum samples AFB negative
Total culture positive (n=334)	118 (35%)	216 (65%)

The age of the NTM infected patients showed that the older age has a higher risk to get NTM infection. The age distribution of NTM cases is shown in Figure 1. The age distribution of NTM infection is 6% in the age less than 20 years old, 23% in age 20-35 years old, 31% in age 36-50 years old, and 40% in age more than 50 years old. It is showed that 72% of NTM cases have been diagnosed in aged older than 35 years.

4. Discussions

NTM are present in the environment, mainly in water, and are occasionally responsible for opportunistic infections in [9]. The frequency of pulmonary disease from NTM is reportedly on the rise in different parts of the world [10]. The clinical and radiologic manifestations of NTM infection frequently overlap with pulmonary TB [10]. This is because the clinical manifestations of NTM infection are often nonspecific [11]. The symptoms are, such as a chronic cough, increased sputum production, dyspnoea, low-grade fever, malaise, and weight loss, and overlapping clinical characteristics with pulmonary tuberculosis [12]. The radiography, clinical sign, and symptom of NTM infection are often
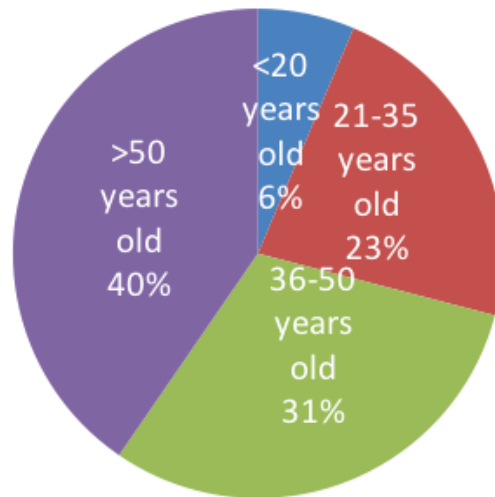
Figure 1: The age distribution of patient with tuberculosis or with presumptive pulmonary tuberculosis infection who were positive for NTM. It is showed that NTM infection was found in older age, 71% of NTM positive were found in patients older than 35 years old.

similar to those due to *Mycobacterium tuberculosis* infection [12]. In addition laboratory diagnostic to detect the NTM lung diseases is difficult and time-consuming due to the bacterial slow growth. These result in the possibility of NTM infection to be misdiagnosed as tuberculosis [11], especially in high burden country of tuberculosis.

The present study showed that there is an unexpected number of NTM infection in Surakarta, Indonesia. Indonesia is a country with the second highest prevalence of TB, but the data about NTM infection is very limited. This study showed that 15%. The data revealed that in five year period there was a consistent number of NTM infection among tuberculosis patient and in tuberculosis suspected patients (11-17%), whereas recently, data in 2017, there was a significant increase (25%) of NTM which is detected in patient's sample with tuberculosis symptoms. The increase of NTM infection was also reported in several countries both in low burden tuberculosis and in high burden tuberculosis countries. In low burden tuberculosis countries, the incidence of pulmonary diseases caused by NTM increases and surpass tuberculosis infection incidence [13, 14]. In high burden countries, such as India, the incidence of NTM infection is also increasing [15], Iran [10], Mali [16], and Zambia [17]. The prevalence of NTM infection in Zambia is 15% for NTM only and 0.2 % were MTB/NTM co-infected [17]. A study in Iran, out of 410 consecutive strains isolated from suspected tuberculosis subjects, 15.1% of them were identified as NTM [10].

Factors that may increase the incidence of NTM infection are pre-existing pulmonary disease, chronic obstructive pulmonary disease, past and/or present history of TB, diabetes, steroids, and malignancy [15]. The increase of individual with HIV infection/AIDS

may also increase the dissemination of NTM infection [15, 18]. In this study, for 5 years period, the prevalence of the detected NTM in patients samples were 15% on average, however, the real number may be higher than this. It is because the detection of NTM still based on conventional methods that are the detection of AFB in sputum samples, growth in LJ=PNB, slow growth colonies in culture, and biochemistry test such as niacin test. More accurate techniques can distinguish into species levels, such as the polymerase chain reaction technique, MALDI-TOF, or other specific techniques.

In addition, since there is a high number of a tuberculosis infection incidence in Indonesia, an infection caused by NTM is still neglected. Moreover, the risk to get NTM infection for tuberculosis patients is high. NTM pulmonary disease often goes unrecognized and is misdiagnosed as pulmonary tuberculosis since the clinical symptoms of these two diseases are similar [15]. The failure to identify NTM lead to the miss diagnosis and treatment; this result in many of NTM infections is considered as multidrug-resistant tuberculosis infection. A study in China showed that 30% out of suspected MDR tuberculosis were NTM infection [19]. It has been also reported that many NTM infections were provisionally diagnosed as pulmonary or extrapulmonary tuberculosis based on clinical presentation and smear microscopy findings [15].

5. Conclusions

The data showed that there was a high number of NTM infection detected in patients with a presumptive diagnosis of TB. The quite high prevalence of NTM infection is unexpected since this disease is usually been neglected. In addition, this finding showed that the possibility that there is undetected NTM infection in tuberculosis patients is may be occurred because of the limitation of the detection method of NTM and the still high concern in tuberculosis infection. Therefore, an approach should be conducted to provide established and accurate detection method of NTM infection and to provide effective therapy for infection due to NTM. Identification of NTM in species level will be important to provide empirical therapy for this disease.

Acknowledgment

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