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The frequency of non tuberculosis mycobacteria among isoniazid resistant Mycobacterium isolates and the pattern of resistance to antituberculosis drugs

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ABSTRACT

Non tuberculosis mycobacteria (NTM) could be the causative agents of various clinical infections, especially in immunocompromised individuals. However, frequency of this bacteria among Mycobacterium isolates is not ye known. Morover, their pattern of resistance to antituberculosis drugs has not been reported. The aim of the study is to determine the frequency of NTM among isoniazid resistant Mycobacterium isolates and the pattern of resistance to antituberculosis drugs. The clinical isolates of isoniazid-resistant mycobacteria collected from several laboratories in Java were cultured on Lowenstein Jensen (LJ) medium and followed by drug sensitivity testing. Identification of NTM was based on standard microbiological test: colony morphology, duration of growth, acid fast staining, and biochemical test (niacin test and nitro benzoic acid medium test). All isolates were resistant to rifampicin, 81.20% were streptomycin resistant, and half of them were resistant to ethambutol. Pattern of resistance to the second line antituberculosis drugs (ciprofloxacin, kanamicin, and ofloxacin) was variable with the range of 37,5% to 62,5%. In conclusion, almost all NTM isolates were resistant to rifampicin and streptomycin, whereas more than half of isolates were resistant to the second line drugs (ciprofloxacin, kanamicin, and ofloxacin).

Key words: NTM- isoniazid resistant- pattern of resistance – antituberculosi drugs

INTRODUCTION

Non tuberculosis mycobacteria (mycobacteria other than *Mycobacterium tuberculosis* and *M. leprae)* are commonly free-living organisms in the environment. They live in surface water, tap water, soil, animals, milk, and food products. The non tuberculosis mycobacteria can also inhabit skin or secretions without causing disease. Recently NTM become important in human infections, since the number of immunocompromised individuals is increasing, particularly those with acquired - immunodeficiency syndrome (AIDS). In broad terms, NTM is divided into 2 groups i.e. mycobacteria causing tuberculosis-like disease (*M. kansasii, M. avium-intracellulare* complex, *M. scrofulaceum*), and those causing soft tissue infections (*M. fortuitum* complex, *M. marinum, M. ulcerans*).[1-5]

Several syndromes are caused by NTM. In children, the most common of these syndromes is cervical lymphadenitis caused by *M. avium* complex (MAC) (including *M. aviumis* and *M. intracellulare*), *M. scrofulaceum, M. fortuitum, M. kansasii,* and *M. marinum.* Less common infections are cutaneous infection, osteomyelitis, otitis media, and pulmonary disease. Disseminated infections are almost always associated with immunodeficiency which is characterized by impaired cell-mediated immunity, such as congenital immune defects or human

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immunodeficiency virus (HIV) infection.[6] Endogenous infection in HIV patients is usually caused by MAC. *Mycobacterium fortuitum, M. chelonae,* and *M. abscessus* that are referred to as "rapidly growing" mycobacteria can be identified in the laboratory within 3 to 7 days. Rapidly growing mycobacteria occasionally have been implicated in wound, soft tissue, bone, pulmonary, and middle-ear infections. Manifestations of disseminated NTM infections depend on the species and route of infection those include fever, night sweats, weight loss, abdominal pain, fatigue, diarrhea, and anemia.[6,7]

Mycobacterium avium complex in the respiratory or gastrointestinal tract is common in persons with HIV infection. Non tuberculous mycobacteria, especially MAC, also can be recovered from 10% to 20% of adolescents and young adults with cystic fibrosis. The usual portals of entry for NTM infection are believed to be abrasions in the skin (eg., for the cutaneous lesions caused by *M. marinum*), the oropharyngeal mucosa (the presumed portal for cervical lymphadenitis), the gastrointestinal or respiratory tract for MAC, and the respiratory tract (including tympanostomy tubes) for otitis media and rare cases of mediastinal adenitis and of endobronchial disease. Most infections remain localized at the portal of entry or in regional lymph nodes. Severe pulmonary disease and dissemination to distal sites primarily occur in immunocompromised hosts, especially in persons with AIDS. No definitive evidence for person-to-person transmission of NTM. However, cluster of patients with the same *M. avium* strain has been reported and may represent a common environmental exposure or person-to-person spread. Cases of otitis media caused by *M. abscessus* have been associated with use of contaminated equipment and water. A waterborne route of transmission has been suspected for MAC infection in immunodeficient hosts.[6-9]

National wide survey covering 32,000 Mycobacterium isolates in USA from 1979 to 1980 revealed that approximately one-third of mycobacterial infection were caused by NTM. The most commonly recognized species were MAC (61%), M. *fortuitum* complex (19 %), and *M. kansasii* (10 %).[1,7,8,11,12] According to the American Thoracic Society, recommendation for MAC lung disease management that the initial therapy for patients consist of a minimum three-drug regimen of clarithromycin (or azithromycin), rifampin (or rifabutin), and ethambutol. In addition, intermittent streptomycin for the first 2 to 3 months of therapy is recommended for extensive disease. Recent randomized trial revealed that a better microbiological response is observed in patients treated with the regimen including streptomycin. For the treatment of *M. abscessus* lung disease, combined intravenous antibiotic therapy including amikacin and cefoxitin for 2-4 weeks for clinical and microbiologic improvement was recommended in addition to the oral antibiotics, including clarithromycin or azithromycin.[13]

In Indonesia the frequency of disease due to NTM has not been reported. The study was conducted to observe the frequency of NTM among isoniazid resistant Mycobacterium isolates and to evaluate the pattern of resistance to antituberculosis drugs.

MATERIALS AND METHODS

Specimen collection

Clinical isolates of isoniazid resistant mycobacteria were collected from several laboratories in Java: BPLK (Balai Pengembangan Laboratorium Kesehatan) Surabaya, BKPM (Balai Kesehatan Paru Masyarakat) Surakarta, BKPM Yogyakarta, RS Persahabatan Jakarta, BPLK Bandung, and Microbiology Laboratory Faculty of Medicine, Gadjah Mada University. The isolates were cultured on Lowenstein Jensen (LJ) medium for 3-6 weeks. The study has been approved by the Health Research Ethics Committee of Faculty of Medicine, Gadjah Mada University, Yogyakarta.

NTM identification

Non tuberculosis mycobacteria was identified based on standard microbiological test i.e. colony morphology, duration of growth on LJ medium, acid fast staining, and biochemical test (niacin test and PNB medium test) as described follow :

Niacin test: 3-4 weeks old culture of mycobacteria on LJ was added with sterile destilled water scarp off the surface growth. As much as 600 uL of the extract were removed and transfered to the test

tube. One niacin strip was added (BBL"Taxo"TB Niacin Test Strip), then the tube was shook gently. After 12-15 minutes, it was compare to the negative and positive control extract. Positive result could be observed by the yellow color of the liquid, and negative reaction was observed if no color change of the liquid.

PNB testing: As much as 100 uL of sample were inoculated on LJ medium which cotains para nitro benzoic acid (PNB) then incubated for at least 4 week. Only NTM can grow on the medium whereas *M. tuberculosis* was inhibited by PNB.

Drugs Sensitivity Testing

Antimycobacterial susceptibility test was performed by the proportional method as described elsewhere. In brief, $5x10^8$ cfu/mL microbial suspension was prepared according to the Mc Farland No 1 turbidity standard and diluted 1:10. As much as 0.2 ml of the diluted microbial suspension was inoculated to LJ medium with or without antimycobacterium drugs. The culture tubes then were incubated at 37°C and growth was monitored after 3 weeks of incubation. Clinical isolates were considered resistant to the antimycobacterium drugs when bacteria growth in the presence of 1 µg/mL of isoniazid, 40 µg/mL of rifampicin, 2 µg/mL of streptomycin, or 2 µg/mL of ethambutol.

RESULTS

Ninethyseven samples of isoniazid resistant mycobacteria were collected during this study. Differentiation of NTM and their susceptibility test result are presented on TABLE 1 and 2. TABLE 1 showed that 16.4 % of 97 isolates of mycobacteria isoniazid resistant were NTM. Most of NTM (40%) isolates were from Bandung.

TABLE.1 Frequencies of isoniazid-resistant NTM according to origin of the isolates

Origin of isolates	Number of INH resistant isolates	NTM isolates	%
Jogjakarta	32	7	21.8
Surakarta	25	2	8.0
Jakarta	10	2	20.0
Surabaya	20	1	5.0
Bandung	10	4	40.0
Total of isolates	97	16	16.4

The susceptibility pattern of NTM isolates to anti tuberculosis drugs were shown on TABLE 2. Almost all of the isolates were resistant to rifampicin and streptomycin and half of them were resistant to etambuthol. No isolate susceptible to first anti tuberculosis drugs. Pattern of resistance to the second line anti tuberculosis drugs was varied, with the range of 37.5% to 62.5%.

Susceptibility test results showed that most isolates were resistant to both first and second line anti tuberculosis drugs. Previous studies reported that susceptibility of individual NTM to antibiotics vary considerably. Susceptibility test of NTM should involve many different antibiotics other than anti tuberculosis drugs, including that sefalosporine and macrolide groups.[14,15]

Among 81.2% isoniazid- resistant isolates in this study, those were also resistant to rifampicin. In term of definition of MDR-TB that *M.tuberculosis* should be at least resistant to isoniazid and rifampicin, if the routine culture and drug sensitivity testing of mycobacteria do not include NTM testing it is possible that bias interpretation of the result may occur. In other words, NTM could be misdiagnosed with MDR-TB.

TABLE 2. Susceptibility profiles of INH resistant NTM isolates

No	Resistant to first line drug				Resistant to second line drug		
	INH	RIF	SM	EMB	CIP	KM	OFLO
1	R	R	S	S	S	S	R
2	R	R	R	R	R	R	R
3	R	S	R	S	R	R	S
4	R	S	R	S	S	S	S
5	R	S	R	R	R	R	S
6	R	R	R	R	R	R	R
7	R	R	R	R	R	R	S
8	R	R	R	S	R	S	S
9	R	R	R	R	S	S	R
10	R	R	R	R	S	S	R
11	R	R	R	S	R	S	R
12	R	R	R	R	S	S	S
13	R	R	R	S	R	S	R
14	R	R	S	S	R	S	R
15	R	R	R	R	S	R	S
16	R	R	R	S	R	S	R
resistant (%)	16 (100)	13 (81.2)	14 (87.5)	8 (50.0)	10 (62.5)	6 (37.5)	9 (56.2)

Notes:
RIF: rifampicin, INH: isoniazid, SM: streptomycin, EMB : ethambutol, CIP: ciprofloxacin, KM: kanamycin, OFLO: ofloxacin, R: resistance, S: sensitive

DISCUSSION

Like in many other countries, the frequency of NTM in lung infection cases in Indonesia is unknown. The reasons of this is that the diagnosis of tuberculosis is mainly based on clinical presentation and direct microscopic examination of sputum. Acid fast bacilli staining and conventional culture can not be used to differentiate NTM from *M. tuberculosis*. Unfortunately, identification of NTM needs several tests (such as niacin, PNB, and catalase test), however the test unable to identify the species of mycobacteria. Species identification of NTM requires complicated procedures. Nucleic acid detection using specific probes provide more accurate identification. Susceptibility testing for NTM should be performed after recognizing the species of mycobacteria. However, this method is not recommended for routine examination, especially in low income countries. To overcome this problem the development of simple species identification technique for NTM is needed.

CONCLUSION

Almost all NTM isolates were resistant to isoniazid, rifampicin and streptomycin, whereas more than half of isolates were resistant to the second line drugs (ciprofloxacin, kanamicin, and ofloxacin).

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