DETECTION OF NTM IN ISOLATES FROM SUSPECTED TUBERCULOSIS USING PCR IS6110 GENE

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ABSTRACT

Introduction: NTM is a group of mycobacterium except M.tuberculosis complex and M.Leprae, which found in the environment and can be pathogenic. Objectives: This study aims to detect NTM isolates using PCR on target genes IS 6110. Methods: This study conducted in Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University and Laboratorium microbiology in Hasanuddin University Hospital. 14 sample isolates of NTM from sputum of suspected tuberculosis patients. The method using PCR IS6110 gene. Results: The results showed that of 14 samples, 8 positive samples with M. tuberculosis and 6 samples negative. Conclusions: IS6110 PCR can be used to detect NTM isolates from sputum of suspected tuberculosis patients.

Key words: NTM, PCR, IS6110, M.tuberculosis
Introduction
Mycobacterium genus consist of more than 140 species which divided into 3 main group of M.tuberculosis complex (MTBC), M.leprae, and Nontuberculous mycobacteria (NTM) (1). Mycobacteria infection is the cause of most of disease in human. NTM infection rise considerably especially in situation where the patients suffered immunodeficiency and using contaminated medical equipments (2). It is also found in immunosuppressed patients (cancer and transplantations) and in people with nonimmunosuppressed condition but with supporting factor for mycobacteria infection such as pollution and cigarette smoke which is the main cause of pulmonary infection (3).

The high prevalence of tuberculosis is influencing the prevalence of NTM infection, especially in developing country. Unlike tuberculosis, most of the countries did not reporting their NTM infection prevalence (4). The lack of data about NTM infection was because the focus of many studies was done in many places with low prevalence of tuberculosis (5).

Aliyu et al., (2013) found that NTM infection in tuberculosis endemic areas in Nigeria (69 isolates from 444 samples which shows tuberculosis infection) (6). NTM cases in tuberculosis area was also found by Da Costa (2013) with 249 isolates from 1580 patients in Para Brazil. Indonesia was ranked 6th from 22 High Burden City (HBC) (7). Data about NTM infection cases in Indonesia was rarely reported. The studies done by Putra et al.(2004), from 23 clinical samples, found 13 positive samples of NTM which is resistant to some kind of OAT (8). This study was done by Msnadckry and Suwardo (2006) to 2.052 sputum from 2.052 TB patients and in 135 other specimen they found M. chelonae infection (0.37% in Jakarta, 3.39% in Semarang, 2.39% in Surabaya, 5.7% in Jakarta/Bandung) and 1% in Jakarta and all of them was found in pulmonary tuberculosis, while there is also extrapulmonary tuberculosis in Bandung 5.5% (9).

A precise identification of Mycobacterium infection has the main role in clinical management for patients. Molecular diagnosis for direct identification from clinical specimen has been massively produced but still come with expensive and limited methods (2).

Cultural method is still being the Gold standard for diagnosis. Although identification of mycobacteria could be done in solid or liquid medium, identification still takes a lot of time because they still using the conventional biochemical techniques. Some methods was developed to identify mycobacteria faster and accurately. AccuProbe (Gene Probe, San Diego, Californnia) and PCR based on hybridization of Inno-LiPA (innogenetics, Ghent, Belgium) was produced to ease the identification, but they were expensive and hard to be used in many institution with limited trained worker (2).

Nowadays, PCR has been used to detect and differentiate MTCB and NTM for routine laboratory diagnosis. Multiplex PCR, with many gen targets was done to meet the target (2). Infection of NTM and MTB could be checked by using specific sequence target in MTB one of which is IS6610. This study identifies the usage of PCR with IS6610 target to detect NTM from sputum isolates.

Materials and Methods
This study was done in Microbiology Laboratory of Hasanuddin University Medical Faculty and Microbiology Laboratorium in Hasanuddin University Hospital. This study was done in July 2014-January 2015.

Study population was sputum materials acquired from patients which were diagnosed with pulmonary tuberculosis
from LabuangBaji Hospital, Wahidin Sudirohusodo Hospital, and Balai Besar Kesehatan Paru. Study samples were sputum materials which met the inclusion criteria which is tuberculosis suspected patients with positive BTA and have not got any therapy, while the exclusion therapy was patients with negative BTA.

Study Method
Sputum acquired were decontaminated and cultured with bilayer medium for 8 weeks. To differentiate M. tuberculosis and NTM we used Immunochromatographic test (CT) by using 3-4 colony culture which inserted into a tube and then vortexed. 100μl suspension was put inside the well, and wait until 15 minutes. The colony which we suspected NTM from ICT extraction to be taken the DNA by using Genosid method to undergone PCR IS6110 check Primer Forward : 5' CCT GCG AGC GTA GGC GTC GG 3' and Primer Revers : 5' CTC GTC CAG CGC CGC TTC GG 3'. PCR was done for 35 cycles in 90°C for 1 minute (denaturation), 60°C for 1 minute (annealing) and 72°C for 1 minute (extension). Extra extension was done in 72°C for 10 minutes.

Results
Sample characteristics
About 30 samples had positive growth in bilayer medium were undergone ICT examination and found that 14 samples are negative in MTB. Negative MTB samples then suspected to be NTM isolates.

Identification with PCR
Confirmation with ICT was done with PCR IS6110 in negative MTB samples undergone molecular examination using IS6110 primer, showing 8 samples with positive MTB and 6 samples with negative MTB.

![Figure 1. Result of PCR IS6110](image)

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Discussion

Early detection was done using ICT method to differentiate MTB colony and NTM ICT which was done according to detection of antigen MPT64. These antigens are specific antigens which is only produced by MTB complex (10). According to study results which was done to 30 samples, 14 samples was negative in MTB (suspected to be NTM). PCR using IS6110 primer to 14 samples found 8 positive samples of MTB and 6 negative MTB samples. IS6110 is an inserted specific sequences found only in MTB complex. IS6110 was used as diagnostic tools in differentiation NTB complex species to other mycobacteria because of the lack genetic exchange with other mycobacteria species (11). Target amplification of IS6110 and hsp65 were specific and sensitive when used to detect NTM strains such as, M.Chelonae, M avium, M fortuitum, M.marinum, and M.kansasii (12). Bensiet al., (2013, multiplex PCR IS6110 method and PRA hsp 65 were very sensitive and specific compared to NAP/BACTEC460, PNB/LJ and PNB/MGIT with no false negative for MTB nor NTM in the study (2).

Meanwhile, the different result of ICT and PCR could be caused by many factors. According to Park (2009), the decreasing viability of tested bacteria, small amount of isolated antigen, or any mutation in MPT64 could lead to negative results in ICT (13). The other explanation was because the test was done after 8 weeks of incubation. Changtai et al (2013), said that MPT64 protein was one of the main filtrate protein which secreted by MTB complex in the first step (14).

Conclusion and Suggestion

Early examination with ICT found 14 NTM samples. Molecular detection using IS6110 primer found 8 positive NTB samples and 6 negative MTB samples. These results shows that IS6110 could be used in differentiating NTM and MTB.

References


