MOLECULAR ANALYSIS OF rpoB GENE OF *Mycobacterium tuberculosis* IN PATIENTS WITH SUSPECTED PULMONARY TUBERCULOSIS IN SURABAYA

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ABSTRAK

*Tuberkulosis yang disebabkan oleh Mycobacterium tuberculosis masih menjadi masalah kesehatan di Indonesia. Langkah awal penting dalam usaha pengendalian TB maupun MDR-TB adalah penemuan kasus TB secara dini, sehingga diagnosis dini secara cepat dan tepat adalah sangat penting untuk menangani kasus TB secara cepat dan tepat pula. Metode diagnosis molekuler cepat berdasar molekuler gen spesifik Mycobacterium tuberculosis membutuhkan data sekuen genom dari bakteri tersebut.Gen rpoB merupakan salah satu gen yang dapat digunakan sebagai alat diagnosis bakteri yang bertanggung jawab terhadap resistensi obat anti TB rifampicin yang menunjukkan mutasi pada daerah tertentu, dan bervariasi secara geografi. Berdasar hal tersebut perlu diketahui sekuen gen rpoB Mycobacterium tuberculosis.Metode penelitian meliputi ekstraksi dan amplifikasi DNA regio gen rpoB Mycobacterium tuberculosis isolat klinik pasien suspect TB paru, dilanjutkan dengan sekuensing.Diperoleh 10 isolat Mycobacterium tuberculosis dari dahak pasien suspect TB paru. Amplifikasi regio gen rpoB Mycobacterium tuberculosis adalah sebesar 411 bp dan hasil sekuens regio gen rpoB Mycobacterium tuberculosis menunjukkan sekuen nukleotida sama dengan Mycobacterium tuberculosis H37Rv. Dari penelitian tersebut disimpulkan bahwa hasil amplifikasi regio gen rpoB adalah 411 bp, dan sekuen regio gen rpoB tersebut mempunyai homologi sekuen DNA 100% atau conserved dengan Mycobacterium tuberculosis H37Rv.**(FMI 2015;51:110-113)***

Kata kunci: *gen rpoB, rifampicin, Mycobacterium tuberculosis, sekuensing.*

ABSTRACT

*Tuberculosis caused by Mycobacterium tuberculosis is still a public health problem in Indonesia. Important initial step in an effort to control TB and MDR-TB is TB case early finding, so early and accurate diagnosis is very important to deal with TB cases quickly and accurately as well. Rapid molecular diagnostic methods based on molecular specific genes of Mycobacterium tuberculosis require the data from the genome sequence of the bacterium. RpoB gene is a gene that can be used as a bacterial diagnostic tool which is responsible for anti-TB drug rifampicin resistance that showed mutations in certain areas, and varied geography. So the sequences of the ropB gene of Mycobacterium tuberculosis need to be known. Research methods include the extraction and amplification of DNA regions rpoB gene of Mycobacterium tuberculosis clinical isolates from suspected of pulmonary TB patients, followed by sequencing. Ten isolates of Mycobacterium tuberculosis from sputum of patients suspected of pulmonary TB were obtained. Amplification of a region of the rpoB gene of Mycobactrium tuberculosis resulst in a 411 bp and sequences of Mycobacterium tuberculosis rpoB gene region showed the same sequence as the nucleotide sequence of Mycobacterium tuberculosis H37Rv. These studies concluded that the region of 411 bp rpoB gene amplification product has a 100% DNA sequence homology with Mycobacterium tuberculosis H37Rv.**(FMI 2015;51:110-113)***

Keywords: *rpoB gene, rifampicin, Mycobacterium tuberculosis, sequencing.*

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INTRODUCTION

Tuberculosis (TB) is an infectious disease that is still becomes a health problem in Southeast Asia. Indonesia is one among the 22 countries with a high number of Tuberculosis (High Burden Countries), the incidence of TB remains high, ranks third in the world (WHO 2006). The WHO report shows that the population of Indonesia is 244.191.000 with a TB incidence of 167 cases per 100.000 populations and a prevalence rate of 281 cases per 100.000 populations (WHO 2012).

Currently, many accurate methods of diagnosis with high sensitivity and specificity were developed. Rapid molecular diagnosis requires the genome sequence data of bacteria. RpoB gene is a gene that can be used as a diagnosis tool (Adékambi et al 2009) which is responsible for anti-TB drug rifampicin resistance. RpoB gene has conserved and variable region (Adékambi et al 2009). Some researchers in several countries have shown that *Mycobacterium tuberculosis* ropB gene mutation in different countries (Pramma-nanan et al 2008).

Until now, the control of TB in Indonesia is not yet optimal,and recommended by the WHO for implementation of global plan to stop TB (11).A very important first effort to control TB is early and appropriate diagnosis to discover TB cases. Moreover, the accurate and quick diagnosis of TB is an important basis for the quick determination of adequate treatment (WHO 2012). Based on the statement, it is necessary to know the sequence of the rpoB gene of *Mycobacterium tuberculosis* in Indonesia.

MATERALS AND METHODS

The study is an explorative descriptive study. The sample is *Mycobacterium tuberculosis* isolates from the sputum of patients suspected of pulmonary TB. First step was DNA isolation of *Mycobacterium tuberculosis* using Eppendorf, and DNA extraction using DNA extraction Kit (Qiagen). The procedure was written in the brochures. Then, amplification of the region of the rpoB gene of *Mycobacterium tuberculosis* was done by PCR. Four-hundred eleven bp region of rpoB gene fragment was amplified with forward primer rpoB-F :5'TCGGCGAGCCCATCACGTCG-3') and reverse primer rpoB-R: 5'GCGTACACCGACAGCGAGCC3', using a PCR machine (Biorad), through the stages: pre Denaturationone cycle at 98^{o}C for 5 minutes, followed by 33 cycles of denaturation at 96^{o}C for 1 minute, annealing at 59 ^{o}C for 1 minute, extention at 72 ^{o}C for one minute, and one cycle of final extention at 72 ^{o}C for 10 minutes. PCR results were analyzed by electrophoresis on 2% agarose gel concentration with ethidium bromide containing 0.5 mikrog/ml in TBE 1X buffer, based on 100 bp DNA marker. *Mycobacterium tuberculosis* H37Rv was used as positive control. Second is DNA sequencing using DNA sequencer.

Region of the rpoB gene sequence were analyzed using BLAST and ClustalW with *Mycobacterium tuberculosis* H37Rv as a control to be compared sample isolates.

RESULTS

There were ten *Mycobacterium tuberculosis* isolates from sputum of patients with suspected pulmonary TB, and then carried out the detection of *Mycobacterium tuberculosis* rpoB gene. DNA extraction from *Mycobacterium tuberculosis* isolates were amplified by PCR. DNA amplification by PCR fragment is 411 bp region of rpoB gene of *Mycobacterium tuberculosis* (figure 1).

Mycobacterium tuberculosis sensitive to rifampicin showed rpoB gene sequence was same with *Mycobacterium tuberculosis* H37Rv, and no DNA mutation in the gene region as shown in figure 2.

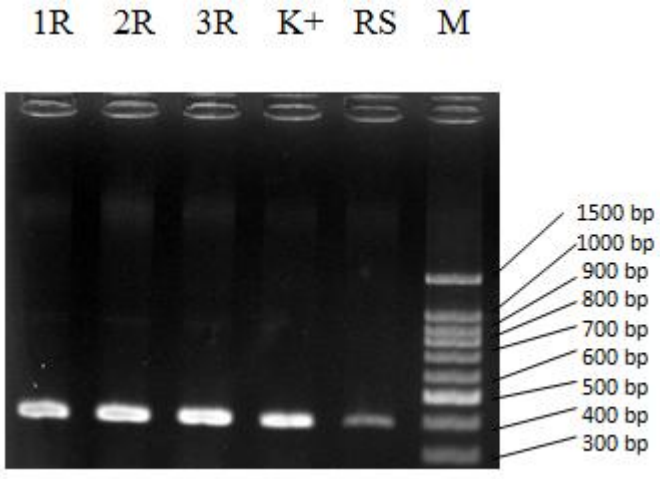

Figure 1. *Mycobacterium tuberculosis* DNA amplification product of 411 bp. (M). DNA marker, (1),(2),(3),(4) isolates from patients samples (K+). *Mycobacterium tuberculosis* H37Rv (positive control) DNA Sequencing.

Figure 2. rpoB gene sequence alignment of *Mycobacterium tuberculosis* isolates against *Mycobacterium tuberculosis* H37Rv.

DISCUSSION

Detection of *Mycobacterium tuberculosis* using specific gene marker is high sensitive and specific. RpoB gene of *Mycobacterium tuberculosis* analysis by PCR targeting 411 bp fragment and sequencing performed. Analysis of the DNA sequence of rpoB gene region has the same DNA sequence with the rpoB gene of *Mycobacterium tuberculosis* H37Rv, which means it does not show a mutation in the region of the gene, so that it can be said the same sequence homology (100%) or conserved with reference strain *Mycobacterium tuberculosis* H37Rv. β subunit of RNA polymerase is a drug target of rifampicin in *Mycobacterium tuberculosis*, which could interfere with the transcription of the DNA that will cause inhibition or death of the bacteria so that the bacteria are sensitive to rifampicin (Abdelaal et al 2009). If there is a mutation in the region of the rpoB gene, resulting in *Mycobacterium tuberculosis* resistant to rifampicin (Choi et al 2010, Palomino et al 2007). Many mutations occurred in hotspot area in rpoB gene of *Mycobacterium tuber-culosis*. Previous research also suggests that the region of the rpoBgene of *Mycobacterium tuberculosis* isolates were sensitive to rifampicin without mutation (Hauck et al 2009, Prammananan et al 2008, Zaczec et al 2009).

CONCLUSION

Amplification region of the rpoB gene of *Mycobacterium tuberculosis* was 411 bp, and the sequence of that rpoB gene of *Mycobacterium tuberculosis* is a conserved sequence.

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