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Characterization of *rpoB* and *katG*: study of RIF resistance to the *subunit-β RNA polymerase* and INH resistance in *katG* gene in patients with TB in Jayapura-Papua Province, Indonesia

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ABSTRACT

The main problem that continues to increase in the treatment and control of TB in the province of Papua is multidrug-resistant of M. tuberculosis (MDR-TB) isolates, which is defined by the World Health Organization, WHO as M. tuberculosis isolates were resistant to RIF and INH. Treatment of TB patients are usually performed by administering three types of anti-tuberculosis medicines with primary choice is rifampin (RIF) and isoniazid (INH), then accompanied with streptomycin or pyrazinamide. RIF resistance due to mutations in the rpoB gene, the gene that produces RNA polymerase β-subunit and INH resistance is largely due to mutations in the gene katG. With the increasing number of people with HIV/AIDS cause TB disease WHO categorizes as a re-emerging disease. Objectives of this study was to obtain information MDR-TB relations with the relevant genes, as well as information combined genotype of M. tuberculosis. Here, we showed that the majority of MDR-TB isolates are resistant to other anti-tuberculosis drugs, and the frequency of mutations rpoB526 and rpoB531 almost the same but katG315 mutation is only found in 16 isolates. This study success to detect mutations in addition to codon rpoB526 or rpoB531 which has never been published, among them the mutation C1307T (Asp516Gly), T1374A and A1376C (Ser539Thr), and C1413T (Pro552Ser). Their C1363A nucleotide changes (Pro535His) in M. tuberculosis sensitive six anti-tuberculosis drugs showed entire rpoB mutations causing resistance properties. On the basis of this phenomenon, it can be suggested that the formation mechanism of MDR-TB strains begins with rpoB mutations followed by mutation katG. This study shows that the mechanism of resistance to an anti-tuberculosis drug that only affects a single gene, such as rifampin affecting rpoB, will be more easily controlled than anti-TB drugs that affect multiple genes, for example isoniazid affecting other genes other than katG.

Keywords: Characterization, RIF and INH, *rpoB* and *katG* gene, resistance of subunit-β RNA polymerase, Papua Province of Indonesia

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INTRODUCTION

Tuberculosis is characterized by tissue death (necrosis) caused by delayed-type hypersensitivity, namely the phagocytosis and presentation of epitopes (antigen recognition) by macrophages in cell surface resulting in a series of processes that trigger reactions of T lymphocytes cells TB Treatment usually performed by administering three types of anti-tuberculosis medicines with the main option is rifampin (RIF) and isoniazid (INH), then accompanied with streptomycin or pyrazinamide. RIF resistance due to mutations in the rpoB gene, the gene that produces RNA polymerase subunit-β and INH resistance is largely due to mutations in the *katG* gene [1].

Research related to MDR-TB has more to do with the nature of bacterial resistance. Due to a mutation of *rpoB*, particularly in the hotspot or RRDR (rifampin resistance-determining region), then RIF can not inhibit the action of RNA polymerase because it can not bind subunit-β, causing resistance to RIF. Meanwhile, INH requires activation process by the catalase-peroxidase enzyme produced by *M. tuberculosis*. Most of INH resistance occurs due to mutations in *katG* gene, the gene that produces the enzyme catalase-peroxidase, so that INH can not be converted into an active form. Until recently identified *katG* mutation causes resistance mutation at codon 315. just a small percentage of INH resistance can occur because of a gene mutation *inhA*, *ahpC*, and *kasA*, as well as other genes that correlated [2-5].

Data show the research results show in Papua province, Indonesia, more than 95% of RIF-resistant *M. tuberculosis* is caused by mutations in *rpoB* and 60-70% INH resistant M. tuberculosis is caused by mutations in *katG*. Some publications also mentioned *M. tuberculosis* isolates that are resistant to RIF phenotype or genotype INH but there are mutations in the *rpoB* or *katG* gene [6-7].

MATERIALS AND METHODS

Isolation and identification

Isolation of *M. tuberculosis* performed with the culture method using Löwenstein-Jensen medium. The media were inoculated and then incubated at 37 ºC for 4-6 weeks or until you see the growth of the colony. Colonies that grew *M. tuberculosis* can not be confirmed due to other mycobacteria can grow well on this medium. Therefore, having seen the growth of colonies to be identified by biochemical reactions, ie by observing the growth and form colonies, test niacin (*nicotinic acid*), catalase test and nitrate reduction test [8].

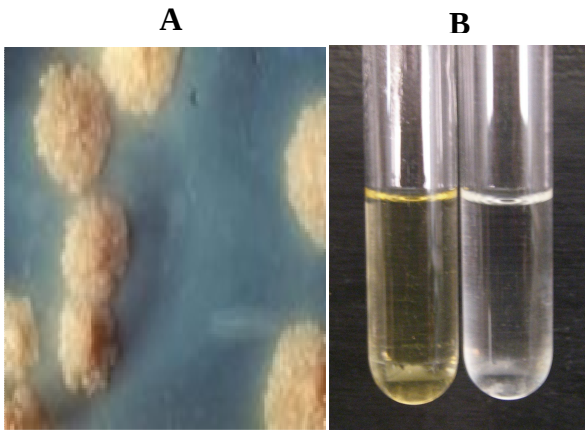

Fig 1. (A) The culture of *M. tuberculosis* Löwenstein-Jensen medium, colonies of *M. tuberculosis* aged 3-4 weeks to grow on the media; (B) The test results niacin *M. tuberculosis*. Test niacin is the main biochemical reactions that must be done for the identification of *M. tuberculosis*

Characterization of phenotype

After obtaining isolates of *M. tuberculosis*, the next step is to test the resistance against six types of anti-Tb drugs. This test is performed by proportional method using Löwenstein-Jensen medium. The suspension isolates are made by inserting a colony of *M. tuberculosis* into a sterile tube containing saline and Glassbead to reach no.1 McFarland turbidity. The suspension was then diluted to 10x and 100x. 0.5 mL bacterial suspension from 10x dilution is taken and put in a test medium, namely media Löwenstein-Jensen slant that has been containing anti-Tb which vary tabunganya. Control is made by inserting a bacterial suspension of 10x and 100x dilution of 0.5 ml into the media control (not containing anti-Tb). The cultures were incubated at 37 °C for 4-6 weeks [9-10].

Characterization of genotype

Characterization of genotypes is based on an analysis of four genes of *M. tuberculosis*, the two genes that produce membrane protein and the other two are the *rpoB* and *katG* genes that cause the nature of *M. tuberculosis* resistant to INH and RIF. Characterization of *rpoB* and *katG* preferred genotype at codon *rpoB526*, *rpoB531*, and *katG315* using multiplex PCR and determining the nucleotide sequence, while analysis on *efpA* and Rv1877 only use the method of determining the nucleotide sequence. Analysis *efpA* gene, Rv1877, as well as segments of *rpoB* and *katG* performed with the method of determining the nucleotide sequence based on the amplification primer pair. In addition, analysis of *rpoB* and *katG* performed by multiplex PCR method that uses three primary, namely the forward primer, reverse primer, and inner primer. Primer is used to detect mutations in codon *katG315*, *rpoB531*, and *rpoB526* thus generated by the primer segments within segments of DNA called a variable. The results of

amplification primer pairs are called non-variable band because there must always be to mark the passage of multiplex PCR process.

RESULTS AND DISCUSSION

After phenotypic characterization of 42 isolates were MDR-TB, then performed a variety of further tests to determine the characteristics of the genotype, ie the multiplex PCR method and determination of nucleotide sequences. Characterization of *rpoB* genotypes aimed at RRDR, mainly codons *rpoB526* and *rpoB531*, characterization *katG* only aimed at codon *katG315*.

Characterization of *rpoB* and *katG* based on the determination of the nucleotide sequence

Analysis of determining the nucleotide sequence performed using band segments of DNA amplification product primer forward and reverse on multiplex PCR. The primer pair amplifying the band length of approximately 0.25 kb and 0.44 kb, respectively for the *rpoB* and *katG*. Primer *rpoB* nucleotide flanking region between 1521 and 1730, or between codons 507 and 576. Thus, a *hotspot* for nature resistant to rifampin or also called RRDR located between codons 507 and 533 amplify entirely. Meanwhile, the determination of the nucleotide sequence of *katG* segment bounded by the nucleotide sequence of 675 and 1104. amplified *rpoB* and *katG* segments can be seen in the picture below (**Fig. 2)**.

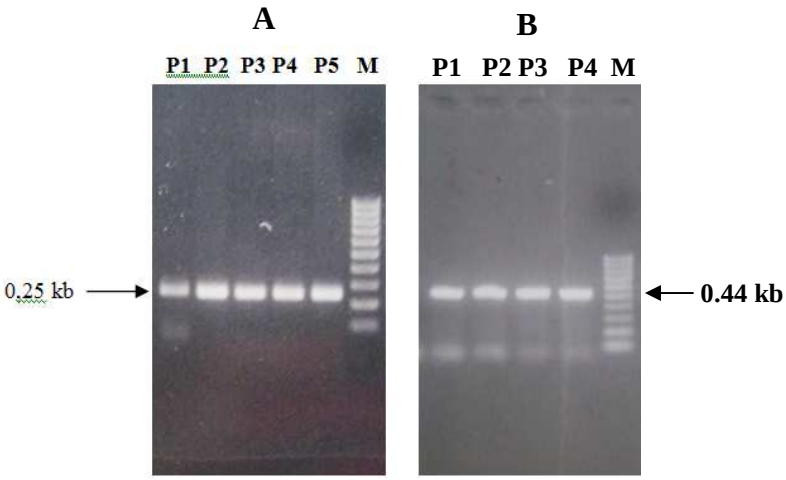

Fig. 2. (A) amplified *rpoB* fragment of 0.25 kb size. Multiplex PCR results are dubious in some isolates, such as P1, P5, and other isolates will be confirmed by determining the nucleotide sequence. After reaching a sufficient concentration, along the 0.25 kb fragment is then used to determine the nucleotide sequence; (M) band marker 100 pb; (B) Results fragment amplified *katG* size of 0.44 kb. Multiplex PCR results are dubious in some isolates, and other isolates will be confirmed by determining the nucleotide sequence. After reaching a sufficient concentration, along the 0.44 kb fragment is then used to determine the nucleotide sequence; (M) band marker 100 pb

Electropherogram analysis results starting with the nucleotide sequence determination focused on nucleotides 1578-1580 (*rpoB526*) and nucleotides 1593-1595 (*rpoB531*). Some isolates detected a mutation in codons with multiplex PCR method, also appear to provide the same results in the electropherogram.

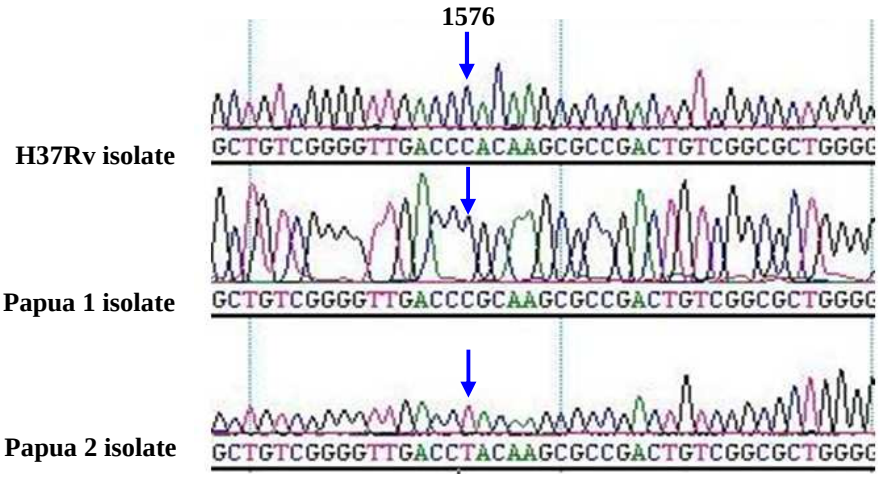

Fig. 3. Electropherogram of *rpoB* some isolates of MDR-TB. On the graph shows the change in the nucleotide isolates P1 and P2 compared with standard isolates of *M. tuberculosis* H37Rv. Mutations are C1576T (isolates P2), and A1577G (isolates P1). Analysis using DNAstar program, with applications of Seqman

Electropherogram results of the determination of the nucleotide sequence of *katG* segment on MDR-TB isolates showed different results with multiplex PCR. Isolates amplify two DNA segments band on multiplex PCR showed no mutations in the *katG315* or nucleotide sequence, *944*. Similarly, isolates the non-variable amplifying the band alone, showed mutations in the nucleotide sequence of 944, ie codon *katG315*. Isolates P4 is mutated isolates multisensitif turns out RRDR, namely the nucleotide sequence C1604A (codon *rpoB535*) that alter amino acids: proline into histidine [2,5,11-18]. This research can be developed to analyze mutations associated disease in an individual, organism, and even just on a particular gene [19-25].

CONCLUSION

A total of forty-two MDR-TB are derived from Laboratory of tuberculosis at the Hospital Dok 2, Jayapura-Papua province, Indonesia, used in this study. Most MDR-TB isolates are resistant to other anti-tuberculosis drugs, and the frequency of mutations *rpoB526* and *rpoB531* almost the same but *katG315* mutation is only found in 16 isolates. This study successfully detected of mutations in addition to codon *rpoB526* or *rpoB531* which has never been published, among them the mutation C1307T (Asp516Gly), T1374A and A1376C (Ser539Thr), and C1413T (Pro552Ser). C1363A nucleotide changes (Pro535His) in *M. tuberculosis* sensitive six antituberculosis drugs showed entire *rpoB* mutations causing resistance properties. Mutated isolates rpoB531 have high levels of resistance to rifampin are relatively higher than other codon mutation, whereas mutations *katG315* and *rpoB526* have resistance levels respectively to isoniazid and rifampin are relatively lower. On the basis of this phenomenon, it can be suggested that the formation mechanism of MDR-TB strains begins with *rpoB* mutations followed by mutation *katG*. This study shows that the mechanism of resistance to an anti-Tb drugs affect only one gene, eg, rifampin affecting *rpoB*, will be more easily controlled than anti-TB drugs that affect multiple genes, for example isoniazid affecting other genes other than *katG*.

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