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DETECTION OF RPOB GENE MUTATIONS IN MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

. The development of treatment for tuberculosis infection gives hope for a complete cure, but over time the use of drugs has an impact on the emergence of strains of Mycobacterium tuberculosis that are resistant to drugs, increasing the speed of diagnostics of resistance, it is necessary to develop one of which is a PCR-based molecular method for determining gene mutations that are caused by drug resistance, one of the drug targets used. rpoB is used, the use of rpoB primer as a marker of mutation is expected to have an impact as an alternative to molecular rapid detection. The rpoB band length of 318 bp was suitable for the Mycobacterium tuberculosis group. The results showed that at codon 509 the AGC to AAT change occurred, an amino acid change from serine to Apagarin, a CAG nucleotide change to CCG at codon 510 changed the amino acid glutamine to proline, codon 514 changed the TTC nucleotide to TTG converts the amino acid Phenylalanine to leucine, codon 515 changes the ATG nucleotide to GTG, this change causes the change of the amino acid Methionine to Valine. These changes lead to resistance to Rifampicin

Keywords: Mycobacterium tuberculosis, Resistance , Mutasi gen rpoB

Introduction

Pulmonary tuberculosis (TB) is an infectious disease which is a priority health problem in developing countries including Indonesia, TB (Tuberculosis) is an infectious disease usually caused by bacteria (Mycobacterium tuberculosis), which mostly attacks the human lungs. *(*(1)., (2)., (3).Drug-resistant Mycobacterium tuberculosis undergoes genetic mutations in cell wall formation and cell wall permeability systems that affect drug activity.Mycobacterium tuberculosis, a pathogen that causes tuberculosis, has a thick cell envelope rich in specific lipids consisting of glycolipids and glycans

The molecular target for rifampicin activity and resistance is the bacterial RNA polymerase encoded by rpoB, Mycobacterium tuberculosis may use different metabolic pathways that contribute to drug activity/resistance
(4).
Multidrugresistant (MDR) strains of Mycobacterium tuberculosis, defined as resistant to at least isoniazid and rifampicin, have emerged as a major health threat worldwide. Spontaneous point mutations in various M. tuberculosis genes confer resistance to isoniazid, with the most frequent gene targets being katG ; and resistance to rifampicin is usually due to mutations in the rp geneoB (5).
The State of Indonesia implemented the Mycobacterium tuberculosis identification

procedure using the Rapid Molecular Test method to detect mutations in the rpo B gene, mutations in the rifampicin drug target gene, which must be confirmed with a drug susceptibility test (DST) which requires examination time for the Mycobacteria Growth Indicator Tube (MIGT) method, between 7 days. up to 21 days and the conventional Lowenstein Jensen method takes 6 weeks to 8 weeks.

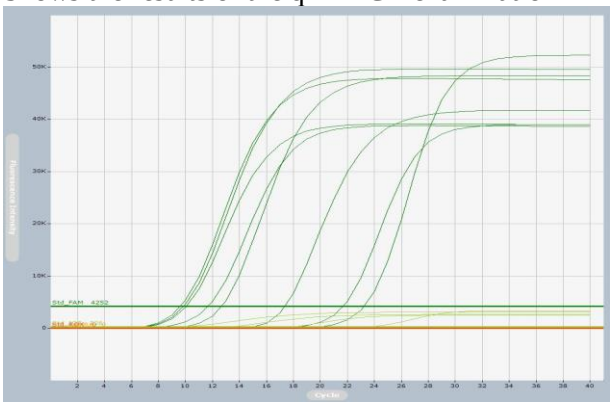
Research methods

This research is a descriptive observational study observing rpoB gene mutations using special primers using the qRT PCR method. Extraction of Mycobacterium tuberculosis DNA using GeneAll reagent with the principle of centrifugation and precipitate by making slight adjustments to the cell lysis process using a temperature of 100 0C for 10 minutes. Optimization of 950C PCR for 5 minutes, 950C denaturation for 15 seconds, 550C aneling for 15 seconds and 720C extension for 30 seconds.

The primer used for the gene rpo B F1 : CGA TCA CAC CGC AGA CGT TG, R1 : GGT ACG GCG TTT CGA TGA AC is located at Start position: 761019 End position: 761336 with length: 318 bp suitable for the Mycobacterium tuberculosis group

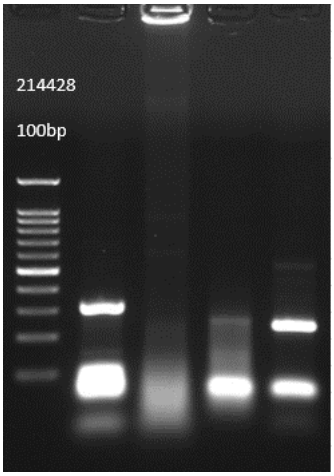
Hasil dan Pembahasan

The results of the study using the rpoB gene specific primer from the test sample Figure 1. Shows the results of the qRT PCR examination



Picture 1 The test results obtained for the H37RV test sample and several clinical isolates that have been identified as Mycobacterium tuberculosis. With a Threshold cycle (Ct) value between 9.62 to 21.65 this provides information that the DNA content at the time of extraction affects the appearance of Ct. High DNA content and specific primer will accelerate the appearance of Ct.

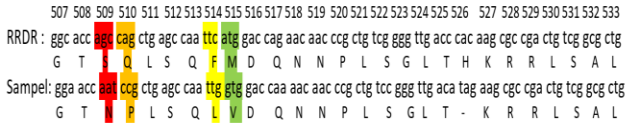
Figure 2 . Electrophoresis results from samples selected for squencing



Picture 2. elektroforesis Result

Dari hasil yang diperoleh uji elektroforesis untuk memastikan apakah hasil PCR yang dilakukan sudah baik. Sampel dilakukan elektroforesis di Genetika Science. Sampel dilakukan sequencing diperoleh hasil, kemudian dilakukan BLAST diperoleh hasil tingkat kesesuain 99,30 % untuk Mycobacterium tuberculosis gen subunit RNA polimerase beta (rpoB). hasil squencing dilakukan pemotongan pada gen RRDR pada gen rpoB sepanjang 81 bp dengan hasil :

gga acc aat ccg ctg agc caa ttg gtg gac caa aac aac ccg ctg tcc ggg ttg aca tag aag cgc cga ctg tcg gcg ctg kemudian dilakukan pensejajaran dengan RRDR untuk mengetahui daerah mutasi pada gen tersebut.



The alignment results in the figure above show that at codon 509 the AGC to AAT change occurred, an amino acid changed from serine to Apargarin, a CAG nucleotide change to CCG at codon 510 changed the amino acid Glutamine to

proline, codon 514 the TTC nucleotide changed to TTG changed the amino acid Phenylalanine to leucine , codon 515 changes the ATG nucleotide to GTG, this change causes the change of the amino acid Methionine to Valine. These changes lead to resistance to Rifampicin.

Mutations of Mycobacterium tuberculosis in the rifampicin resistance determining region (RRDR) in the rpoB gene, mutations in other genes rpoA and rpoC can increase Mycobacterium tuberculosis's resistance to OAT. Ma, et al 2021. Genetic mutations in rpoB mostly occur in Asp441Val, Ser456Trp, Ser456Gln, Arg454Gln, His451Gly, and His451Pro (6)).

M. tuberculosis mutations in the Rifampicin Resistance Determining Region (RRDR) in the rpoB gene can increase M. tuberculosis resistance to OAT ((Fernando et al. 2019); (Ma et al. 2021)(M. C. Li et al. 2021) Genetic mutations in rpoB mostly occurs in Asp441Val, Ser456Trp, Ser456Gln, Arg454Gln, His451Gly, and His451Pro ((Amusengeri et al. 2022)) mutations in S450L, H445D, H445Y and H445R are associated with high RIF resistance with a MIC of 128 ug, whereas D435V is associated with moderate resistance (7)(8). Rifampicin is a strong bactericidal agent for the treatment of M. tuberculosis infection in both log and stationary phases by inhibiting RNA polymerase (RNAP) mechanism. Blocks RNA elongation when transcription is at the 5' end or reduces the affinity of RNAP to short RNA transcripts. Mutations will affect the action of the drug in inhibiting RNA polymerase (RNAP).
(9)

The rpoB gene for the RNA polymerase B subunit, is the target of rifampicin which is present in all bacteria with varying length and sequence between bacterial species. In 1993 the numbering was determined based on the annotation sequence of Escherichia coli (Andre et al. 2017) The genetic mutation of rpoB M. tuberculosis at codons (amino acids) 435, 445 and 450 can be equated with codons 516, 526 and 531 in the E.coli numbering system

Mycobacterium tuberculosis is not like gram-positive or gram-negative bacteria in its life development which is capable of gene transformation. In its development, if Mycobacterium tuberculosis has experienced drug resistance to infect people, then that person will also get an infection with the same resistance. If this happens, it can be concluded from one type of drug resistance, for example Isoniazid (monoresistant), to Multi-drug Resistant (MDR) resistant to Isoniazid. and Rimfamizin, being Extensively Drung Resistant (XDR) which is MDR plus Fluoroguinolone and one of the second-line injection drugs amikacin, kanamycin or rifampin, the most extreme thing will be Totally drung resistant (TDR) all resistant drugs. That's what will happen if not handled properly. Genetic diversity of Mycobacterium tuberculosis by region will provide information on the distribution of these strains and genetic mutations provide information on polymorphism between strains for gene mutation mapping.

Conclusion

The primer used was able to amplify the rpoB target gene in Mycobacacterium tuberculosis with a band length of 318 bp covering an 81 bp RRDR region with mutations at codons 509, 510, 514 and 515 which caused Mycobacterium tuberculosis to be resistant to Rifamphin.

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