Genome Changes in Multi Drug Resistant Tuberculosis

T U Soleha¹, Sutyarso², and A Sukohar³

¹Doctoral Program FMIPA Universitas Lampung, Bandarlampung, Lampung, Indonesia
²Biology Departement FMIPA Universitas Lampung, Bandarlampung, Lampung, Indonesia
³Farmacology Departement FK Universitas Lampung, Bandarlampung, Lampung, Indonesia

¹dr.triumiana.unila@gmail.com; ²sutyarso@yahoo.co.id; ³asepsukohar@gmail.com

Abstract. Directly Observed Treatment Shortcourses (DOTS) is a tuberculosis disease program that has been implemented worldwide since 1995. Prevalenve of Tuberculosis has started to decrease but since then there was a new phenomenon of the resistant Tuberculosis or Multi Drug Resistant Tuberculosis (MDR TB). Indonesia is now a country with a high burden of MDR TB in the world. MDR TB is resistance to Anti Tuberculosis drug that is type Isoniasid and Rifampisin. Usually the cause of resistance is irregularity of taking medication. It is now known that Tuberculosis drug resistance is not only caused by inadequate or failed treatment but also due to the emergence of resistant strains transmitted by MDR TB sufferers. Resistant strains arising from changes or mutations in certain genes in the Mycobacterium tuberculosis genome include rpoB gene and katG gene which are the work targets of Anti Tuberculosis Drugs. This resistance mechanism is strongly associated with the occurrence of a number of changes in the structure of the Mycobacterium tuberculosis genome. Mycobacterium tuberculosis develops resistance mechanisms that are different from other bacteria in general. Therefore this paper is expected to help understanding the genomic changes that occur in the mechanism of resistance Tuberculosis.

1. Introduction

Indonesia is in the top 3 with the highest prevalence of Tuberculosis in the world after India and China. Difficulties in early detection of cases cause an increase in the number of Multi Drug Resistant Tuberculosis (MDR-TB). With the Directly Observed Therapy (DOTS) program, Tuberculosis cases in Indonesia have decreased but increased in the case of resistance. [1] MDR-TB is a condition where the bacterium Mycobacterium tuberculosis has resistance to anti-tuberculosis drugs namely rifampicin and isoniazid (INH) with or without resistance to other drugs. There are 2 types of Tuberculosis resistance which are primary and secondary. Primary resistance is resistance that occurs in patients who have never received anti-tuberculosis drugs before which is usually found in HIV-positive patients. Whereas secondary resistance is resistance obtained during therapy in people who were previously sensitive to drugs. It is estimated that there are 2% of MDR-TB cases of primary resistance and 19% of MDR-TB are cases of secondary resistance in Indonesia [2-3].
In MDR-TB, *Mycobacterium tuberculosis* has mutations to be resistant to one of the anti-tuberculosis drugs due to inadequate drug use or just taking one type of drug. TB patients with secondary drug resistance can transmit to other patients where the patient is said to have primary resistance. This has made it easier to transmit to HIV-infected patients whose immune system has decreased. Drug resistance in TB is not only caused by inadequate or failed treatment but is also caused by the emergence of resistant strains transmitted by MDR-TB patients who have mutations in the genes that are targets of anti-tuberculosis drugs. [4-6]

2. Result and Discussion

The characteristics of slow or dormant growth of *Mycobacterium tuberculosis* are very important in the treatment of TB patients. This situation occurs due to the suppression of bacterial metabolic pathways due to cellular immune system activation. This mechanism is a form of infection defense but does not eliminate the infection itself. Natural resistance to many types of antibiotics is one of the unique *Mycobacterium tuberculosis* bacteria [7].

Various genetic traits in *Mycobacterium tuberculosis* that play a role in resistance mechanisms include cell walls that are highly hydrophobic, a number of determinants in the genome, expression of hydrolytic enzymes such as beta-lactamase and aminoglycosidase acetyl transferase and efflux pump systems. Genetic and molecular analysis of *Mycobacterium tuberculosis* shows that resistance mechanisms are usually obtained through mutations in drug targets [7-8].

There are 2 types of resistance to MDR-TB, namely intrinsic resistance and acquired resistance. Intrinsic resistance is a low level of resistance caused by highly hydrophobic cell wall structures and plays a role in maintaining cell wall permeability from the mechanism of drug destruction. Acquired resistance that occurs in MDR-TB is generally caused by a number of mutations in a number of genes that encode bacterial sensitivity to anti-tuberculosis drugs [8].

Resistance to *Mycobacterium tuberculosis* is triggered by the presence of mutations that occur spontaneously in the chromosomal gene. Resistance will only benefit bacteria if exposed to the target drug. On exposure to anti-tuberculosis drugs that are inadequate sensitive bacteria will die and those who experience mutations will multiply rapidly without competing nutrients. Tuberculosis in the case of MDR will develop into chronic and further facilitate the spread of *Mycobacterium tuberculosis* MDR strains [9].

Anti tuberculosis drug mutations occur $10^{-9}$ times per cell division. Therefore antituberculosis drug is given in combination to minimize the possibility of mutations up to $10^{-18}$ per cell division. A number of genes involved in the mechanism of resistance of *M. tuberculosis* to OAT(stand from ....) can be seen in table 1.

| Drug            | Gene   | Product                                      | Reported frequency of resistance strains (%) |
|-----------------|--------|----------------------------------------------|---------------------------------------------|
| Rifampisin      | *rpoB* | B subunit of RNA polymerase                  | >95                                         |
| Isoniazid       | *katG* | Katalase peroksidase                         | 60-70                                       |
| oxR-ahpC        |        | Alkyl hidroksi-reduktase                     | 20                                          |
| INH-ethionamide | INH-A  | Enoyl-ACP reductase                          | <10                                         |
| Streptomycin    | *rpsL* | Ribosomal protein s12                       | 60                                          |
|                 | *rrs*  | 16s rRNA                                     | <10                                         |
| Fluoroquinolone | gyrA   | DNA gyrase                                   | >90                                         |
| Pyrazinamide    | *pncA* | Amidase                                      | 70-100                                      |
| Ethambutol      | embCAB | embCAB                                       | 69                                          |
*Mutation frequencies are as determined by sequencing and polymerase chain reaction singel strand conformational polymorphism (PCR-SSCP) analysis

The mechanism of resistance to isoniazid (INH) is caused by a mutation in the katG gene that plays a role in encoding the enzyme catalase peroxidase which is useful to activate the INH that enters the body as a pro drug. INH is the hydration of isonicotic acids, molecules that are water soluble so that it is easy to enter the cell. The mechanism of INH resistance is thought to be due to the presence of amino acids that change the catalase peroxidase (katG) gene or promoter in the 2-gene locus known as inhA. Missense mutations or katG deletions are associated with reduced catalase and peroxidase activity. It is assumed that this mutant is the result of a second stage of mutation after INH exposure in suboptimal doses. The mutation that is often found in katG is the Serin amino acid mutation to Treonine in codon 315. This mutation is often found in MDR-TB cases compared to the INH monoresistant [11-13].

Rifampicin plays an active role in gram-positive and gram-negative cocci in the mycobacterium, chlamydia and poxvirus bacteria. This resistance to rifampicin is caused by barrier permeability or the presence of mutations from RNA polymerase. Resistance to rifampicin develops due to changes in RNA polymerase with high mutation rates. Resistance that occurs in genes with beta subunits of RNA polymerase results in changes in the location of the drug binding [11].

The rpoB gene is a gene that encodes the Beta subunits one of the structures that make up the DNA polymerase enzyme that is against rifampicin resistance. Characteristics of rifampicin are the ability to kill bacteria that grow either actively or not. Mutations in the rpoB gene will change the structure of the Beta sub-unit so that rifampicin loses site of action. Mutations generally occur in 81 base pairs in the core area called Rifampicin Resistance Determining Region (RRDR) and are usually in the form of a single nucleotide change that will result in changes in amino acid composition [10,14].

The mechanism of resistance of Pirazinamide is related to the loss of Pirazinaminidase activity so that not much pyrazinamide is converted to pyrazinoic acid. Pirazinamide is a nicotinic acid derivative that plays an important role as a short-term bactericid against tuberculosis therapy [15].

Pirazinamide is a nicotinamide analog that works selectively against M. tuberculosis. This drug is effective in inhibiting the growth of semidominant bacteria that cannot be killed by other drugs and synergistic with rifampicin and INH. Pyrazinamide has sterilization activity at the beginning of therapy which is able to kill persistent bacteria and shorten the therapy period from 9 months to 6 months. The mutations that occur in PncA can be point mutations, insertions, and nucleotide deletions and termination mutations [9,12,16,17].

The mechanism of Ethambutol resistance in M. tuberculosis is related to a missense mutation in the embB gene which is the password for arabinosyltransferase. This mutation occurs in 7% of resistant strains and the replacement of amino acids at position 306 or 406 occurs in about 90% of cases. Ethambutol is one of the first line anti-tuberculosis drugs that works synergistically with other anti-tuberculosis drugs in inhibiting the synthesis of M. tuberculosis cell wall arabinolactant, especially in inhibiting arabinosil transfer. Mutations that greatly affect the phenotype of ethambutol resistance occur in the embB gene. The embB gene has methionine in the codon 306 position. The point mutations that result in the substitution of Methionine amino acids in position 306 are found in most cases of ethambutol resistance [9,10,14].

3. Conclusion
Multidrug resistant to tuberculosis is a global health problem that is not only caused by inadequate treatment but also by the genetic structure or composition of the M. tuberculosis genome which affects the resistance mechanism. Resistance to rifampicin occurs due to mutations in the rpoB gene, isoniazid in the katG and ahpC genes, pyrazinamide in the pncA gene, ethambutol in the embB gene.
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