

Molecular Detection of Dapsone and Rifampicin Resistance on *Mycobacterium leprae* from Leprosy Patients in East Java

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The drug resistant problem of *Mycobacterium leprae* has been developing since the last decade and this has become a leprosy elimination problem in several countries, including Indonesia. Using biological on molecular methods, it is now possible to test for drug resistant cases in relatively simple and less time consuming ways. The purpose of the study is to analyze the prevalence of drug resistance *M. leprae* to dapsone and rifampicin in East Java based on the detection of mutations in the *folP* and *rpoB* genes. All samples were obtained from multibacillary leprosy patients in East Java, who have admitted to the Dr Sutomo Hospital Surabaya in 2003-2005. Isolates were analyzed by PCR, and the presence of nucleotide sequence of the *folP* and *rpoB* genes from *M. leprae* were confirmed by direct sequencing. Of 94 specimens which were collected, all were analyzed for their *folP* and *rpoB* genome. From 94 isolates, 70 showed a positive result by the folP1-folPR test and 77 out of 94 isolates showed positive by the rpoBF-rpoBR test. From 70 isolates for *folP* gene examination, there were 3 isolates which had mutation in the amino acid at codon 53; 2 cases Threonin (ACC) became Alanin (GCC) and 1 case Threonin (ACC) became Arginin (AGA). These mutations are responsible to dapsone resistance. For the *rpoB* gene, no mutation was found. The result suggested that 3 isolates (4.3%), 1 from a new case and 2 from relapse cases in this experiment, were resistant to dapsone and all isolates (100%) were susceptible to rifampicin.

Key words: resistance, *Mycobacterium leprae*, dapsone, rifampicin, East Java

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. The disease mainly affects the skin, the peripheral nerves, mucosa of the upper respiratory tract and also the eyes, apart from some other structures. Leprosy has afflicted humanity since immemorial time. It once affected every continent and it has left behind a terrifying image in history and human memory-of mutilation, rejection and exclusion from society (WHO 2001).

Since the introduction of multidrug therapy (MDT) by the World Health Organizations (WHO) for the treatment of leprosy in 1982, the worldwide prevalence rate of leprosy has significantly decreased (Brosch *et al.* 2000). The MDT regimen was based on rifampicin, clofazimin and Dapsone (DDS). However, rifampicin, ofloxacin and minocycline (ROM) and also clarithromycin were prescribed to patients who refused to take clofazimine fearing skin discoloration (You *et al.* 2004). It is found in only several countries in Africa, Asia and South America, and is mainly present in limited numbers. On the contrary, the number of new cases of leprosy has remained unchanged over the last 10 years. This situation could inhibit our ability to target drug therapy campaigns and to improved control strategies (Williams *et al.* 1994).

Current recommended control measures for treatment of leprosy with MDT are designed to prevent the spread of drug resistant *M. leprae*. However, drug resistant *M. leprae* strains have been reported, and this might become the problem for eliminating leprosy (Maeda *et al.* 2001).

Maeda *et al.* (2001) studied the MDT-resistance from the DNA sequences from particular regions of *M. leprae* *folP1* (Accession no. AL023093), *rpoB* (Z14314) and *gyrA* (Z70722).

All the genes were responsible for resistance to dapsone (DDS), rifampin and ofloxacin respectively. Several *M. leprae* isolates showed point mutations in these genes. These results suggest the need for a multidrug resistance (MDR) study for leprosy.

Kai *et al.* (1999) has shown that substitution of amino acids at 53rd and 55th position in dihydropteroate synthase (DHPS), which are coded by the *folP1* gene, cause resistance against DDS in *Mycobacterium leprae*. Whereas, in *M. tuberculosis* and *M. leprae*, resistance to rifampicin involved mutation in the *rpoB* gene encoding the β -subunit of the RNA polymerase of these species (Williams *et al.* 2000). It was revealed that mutation at the 513, 516, 526, 531, and 533 positions of amino acids confer rifampin resistance (Maeda *et al.* 2001). Jin and Gross (1988) have also shown the same causes for resistance against rifampicin after cloning in *Escherichia coli*.

The East Java province still has the highest number of leprosy patients (3.27 per 10 000 inhabitants), and new cases still occur (2.43 per 10 000 inhabitants), although treatment was undertaken and the patients were all cured (Dinkes Jatim 2008). About 35% of leprosy patients in Indonesia were in East Java (Depkes RI 2006). The present study is to observe the prevalence of drug resistance *M. leprae* for dapsone (DDS) and rifampicin in East Java-Indonesia based on the detection of mutations in the *folP* and *rpoB* genes.

MATERIALS AND METHODS

DNA Templates Preparation. Ninetyfour *M. leprae* isolates were taken from the lesion of multibacillary (MB) patients, categorized based on the criteria of the World Health Organization (WHO). There are 48 isolates (51%)

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