DETECTION OF gyrA GENE MUTATION REGION IN FLUOROQUINOLONE RESISTANT Mycobacterium tuberculosis ISOLATE FROM SURABAYA INDONESIA

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ABSTRACT

Fluoroquinolones are an important class of antibiotics used in the management of bacterial diseases and drug resistance tuberculosis. This study aimed to detect gyrA gene mutations regions as the mechanism of fluoroquinolone resistance in Mycobacterium tuberculosis by sequencing the quinolone resistance determining region (QRDR) of gyrA, one of the genes encoding DNA gyrase. Mutations affecting amino acids at positions 74, 90, 91 and 94 have been most commonly associated with resistance. Drug susceptibility testing of Mycobacterium tuberculosis was performed by the culture method of proportion, including susceptibility to ofloxacin. DNA was extracted by boiling method. Nucleic acid was amplified by PCR, targeting the region of gyrA using primers gyrA-F (5’CAG CTA CAT CGA CTA TGC GA 3’) and gyrA-R (GGG CTT CGG TGT ACC TCA T) and sequenced with ABI 377 sequencer. Sequences were analysed to identify mutations associated with fluoroquinolone resistance. There were 5 ofloxacin resistant Mycobacterium tuberculosis isolates which were PCR processed for gyrA. The amplicons product were 320 bp from five these isolates. No mutations were identified in gyrA specifically at sites corresponding with codons 90, 91, and 94, but all 5 isolates had the mutations S95T. These findings suggest that the gyrA mutation, S95T is suspected as the polymorphism in fluoroquinolone resistant Mycobacterium tuberculosis. However, further investigation is required to identify the mechanism of fluoroquinolone resistance in Mycobacterium tuberculosis. (FMI 2014;50:139-142)

Keywords: fluoroquinolone resistance, Mycobacterium tuberculosis, gyrA, mutation

INTRODUCTION

Tuberculosis requires adequate therapies. Along with the emergence of the phenomenon of resistance to anti-TB, the diagnosis of Mycobacterium tuberculosis resistance to anti-TB drugs, especially fluoroquinolone need attention (Mohapatra 2010). Fluoroquinolone become one therapeutic option for cases of MDR-TB and TB cases with drug induced hepatitis (WHO 2010). Today the use of fluoroquinolone spread in the community for lower respiratory tract infection therapy community acquired pneumoniae (CAP) and urinary tract infection (Chang et al 2010, Moadebi et al 2007). In Dr. Soetomo Hospital 87% of pulmonary TB patients are known to using fluoroquinolone to address the clinical manifestations of secondary infection accompanying TB. This is worrying, proven research about the risks of TB patients have a history of resistance to quinolones are associated with exposure to quinolones (Xu et al 2009). Fluoroquinolone resistance is known to appear at least 13 days after fluoroquinolone treatment. Studies in patients with a history of TB

Kata kunci: Resistensi fluoroquinololin, Mycobacterium tuberculosis, gyrA, mutasi gen

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exposure fluoroquinolone more than 10 days prior to TB diagnosis showed 20.8% had enforced the risk of resistance of *M. tuberculosis* against fluoroquinolone (Devasia et al 2009). Along with the increasing use of fluoroquinolone as respiratory disease drug therapy and other comorbid infections and emerging of MDR-TB and XDR-TB cases, research aimed to detect gyrA gene mutation in fluoroquinolone *M. tuberculosis*-resistant isolate.

**MATERIALS AND METHODS**

Research conducted by the research stages: (1) test the sensitivity of *M. tuberculosis* against fluoroquinolone (ofloxacin) by the proportion method culture in *M. tuberculosis* isolates collected from sputum culture-pulmonary TB patients (2) Extraction of DNA by the method of heating (Boiling) (3) Amplification of nucleic acids using PCR method for the gyrA gene by gene primer gyrA with a primer sequence of gyrA-F (5'CAT CTA CAT CGA TGC GA 3') and gyrAR (GGG CTT CGG TGT ACC TCA T) (Murray et al. 2007). (4) PCR products were then purified and then performed gyrA gene encoding DNA sequencing with ABI 377 Sequencer System Machine. The results were analyzed sequencing DNA mutation using a software program GENETYX win by 9.0 by comparing wild-type *M. tuberculosis* H37Rv from Gene Bank.

**RESULTS**

Description of fluoroquinolone resistant *M. tuberculosis* isolate characters stored in ITD-UNAIR tuberculosis laboratory, 5 isolates showed visible colonies sub-cultured *M. tuberculosis* a creamy white, dry, rough like a cauliflower in the culture medium. From the result of sensitivity testing of *M. tuberculosis* against fluoroquinolone (ofloxacin) by proportion method, proved there 5 isolates were resistant to the antibiotic ofloxacin detected 5-isolates of FQr-TB were DNA extracted and continued amplification of gyrA gene. GyrA gene was detected by PCR technique that shown in Figure 1. All of 5 strain FQr-TB that have been sequenced revealed changing of G-base to C base (Figure2).

**DISCUSSION**

The finding of 5 isolate of ofloxacin resistant-*M. tuberculosis* is in line with the results of a study in India, which reported fluoroquinolone- resistant *M. tuberculosis* (FQr) 71 isolates of 118 isolates were examined (60%), in China 68 of 109 (62.3%) isolates were resistant to fluoroquinolones (Chang et al 2010) and in Thailand found 35 isolates FQr-MTB isolate (Pitaksajjakul et al 2005), more higher than Tanzania 0, 7% (2 of 291) MTB isolates FQr- (van den Boogaard et al 2011), whereas in the two-state Southwest Nigeria, the prevalence of resistance to the details of 7.5% ofloxacin resistant which 4.3% of that found in newly diagnosed TB patients and 9.1% was found in patients TB has been treated, although there was no significant difference ofloxacin resistance patterns in patients with newly diagnosed TB patients with TB treated (Daniel et al 2011).
this study, the results of molecular genetic analysis to detect gene mutations in five isolate of ofloxacin resistant *M. tuberculosis* showed changes in position 284, from G bases turned into base C. This indicates the occurrence of point mutations that lead to changes base and amino acid analysis from Serine to Threonine (S95T). On research Zhenling et al. (2011) stated 8.4% ofloxacin resistant *M. tuberculosis* strains had gyrA gene mutation S95T. The presence of mutations in codon 95 amino acid (Serin-Threonine) indicates the presence gyrA gene polymorphism of *M. tuberculosis*. Polymorphism is associated with tuberculous bacteria group division into three categories: group 1 showing polymorphism in gyrA (Murray et al. 2007, Aubry et al. 2006) ACC and kat G (Moadebi et al. 2007, Demas 2007, Chang et al. 2010), group 2 showed polymorphism gyrA (Murray et al. 2007, Aubry et al. 2006) ACC and kat G (Moadebi et al. 2007, Demas 2007, Chang et al. 2010) CGG, and group 3 gyrA (Murray et al. 2007, Aubry et al. 2006) AGC and kat G (Moadebi et al. 2007, Demas 2007, Chang et al. 2010) GGG. The variation S95T are most often found in some studies (Mohapatra 2010, Aubry et al. 2006).

S95T mutation discovery in this study showed that there is polymorphism of *Mycobacterium tuberculosis*, but mutation in codon 95 is not a marker of resistance. This is supported by sequence analysis conducted by researchers at several isolates of *Mycobacterium tuberculosis* that are sensitive to ofloxacin, also have mutations in codon 95. Matrat et al. (2008) reported that the nucleotide sequences of the isolates were sensitive to ciprofloxacin indicate a change in the amino acid serine at codon 95 is a Threonine, while Mycobacterium strains that are resistant to fluoroquinolones showed that mutations in the conserved region in the 67-106 region known as QRDR (quinolones Resistance Determining Region) in the gyrA gene. Change of point mutations in DNA gyrase subunit area will result in modification of enzyme function (Matrat et al. 2008). Several studies indicate that gyrA gene mutations associated with resistance to fluoroquinolones are known lies in codon positions 88, 90, 91, and 94 (Aubry et al. 2006, Pitaksajjakul et al. 2005). Study was conducted in Bandung on XDR strains showed no gyrA gene mutations at codons 90, 91, 94 (Astuti 2008, Pitaksajjakul et al. 2005) and Zhenling et al. (2011) reported Fqr-MTB isolates (40%) no mutations in codons 90, 91, 94. Siddiqi et al. (2002) explained 88.5% (61 from69 strain) which also showed no mutation at that point, and 3.2% isolates of research in Lisbon Portugal also did not find any mutations in the coding points of hot spots (Perdigão et al. 2010).

Our research on the five isolates were resistant to fluoroquinolones showed no mutations in the hot spots of gyrA gene resistance, it is likely due to the other mechanism such as mutation gyrB gene or efflux pump mechanisms (Pitaksajjakul et al. 2005, Huang et al. 2005). Mechanisms of fluoroquinolone resistant *Mycobacterium tuberculosis* may be due to gyrB gene mutation at position Asp495 His/Asn, Arg516, and Asn 533 N, and N510 and N499D (Aubry et al. 2006, Pitaksajjakul et al. 2005). Other mechanisms of fluoroquinolones resistant *Myobacterium tuberculosis* is due to increased pumping mechanism drugs out of cells (efflux pump). Efflux system is typically capable of causing resistance to the combination of various types chemical structure of drug, as known as multi-drug resistance pumps (MDR pumps) (Setiabudy 2008, Kocagöz et al. 1996). The presence of LfrA gene influence the efflux pump of mycobacteria (Telenti 1998). LfrA protein is a protein group Major Facilitating superfamily (MFS), the second group of the three groups that play a role in bacterial efflux pump. LfrA protein associated with resistance to fluoroquinolones with expulsion mechanism and restricted access to antimicrobials to targets. Other study explain mechanisms of causing of fluoroquinolones resistant *M. tuberculosis* is MtMfpA protein that binds to and inhibits active site of DNA gyrase that result in fluoroquinolone resistant *M. tuberculosis* (De Rossi et al. 2002). This study cannot relate whether this resistance to fluoroquinolones associated with a history of previous treatment, although the known effect of fluoroquinolones may also be the cause of resistance (Devasia et al. 2009).

**CONCLUSION**

Detection of gyrA gene mutation region in fluoroquinolone *M. tuberculosis* resistant isolates showed warning information to clinician about utilizing of fluoroquinolone drug. Five fluoroquinolone *M. tuberculosis*-resistant isolates had no mutation on 90, 91, 94 amino acid that were ‘hotspot’ of fluoroquinolone resistant, but had mutation on 95 position (Serin-Threonin. No connection between fenotipic resistant of Fluoroquinolone with mutation on 95. This mutation showed the polymorphism of *Mycobacterium tuberculosis*.

**REFERENCES**


