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Detections Of *Gyra* And *Gyrb* Gene Of*mycobacterium Tuberculosis* Clinical Isolates Resistant Ofloxacin In North Of Sumatera, Indonesia

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Abstract: *This study aimed to detect mutation of the gyrA and gyrBgene in Mycobacterium tuberculosis resistant ofloxacin with phenotype and genotype test. This study presented by phenotype method with MGIT 960 and genotype method with geneXpert and PCR. The Mycobacterium tuberculosis H37Rv was used as a reference bacteria in this study.Among 42 clinical isolates, the TB-RR, MDR-TB, XDR-TB and Pre-XDR-TB were 42/42 (100%), 41/42 (97,62%), 11/42 (26,1%), and 31/42 (73,90%). All 42 (100%) isolates were ofloxacin- resistant by the phenotype test with MGIT 960 method. In Genotype with gene Xpert, we found that all isolates were MTB detected and RIF resistance detected. In the PCR test, all 42 ofloxacin resistant isolates showed 37/42 (88.09%) mutations in the gyrA gene and 5/42 (11.90%) isolates showed no mutations in the gyrA gene. in the gyrB gene, no mutations were found in the MTB isolates.This study was showed discordance results on 5 isolates, which in phenotype test all isolates showed resistance ofloxacin but in the genotype test, 5 isolates did not show mutations in the gyrA and gyrB genes against mycobacterium tuberculosis isolates.*

Keywords:*Mycobacterium tuberculosis, gyrA, gyrB, ofloxacin,*

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I. Introduction

Tuberculosis (TB) is a disease in humans that can be transmitted through droplets, which are caused by the bacterium *Mycobacterium tuberculosis* (MTB). This remains an important global problem in developing countries that have a high burden of TB case rates, especially TB that is resistant to several types of drugs (MDR-TB) and is widely resistant to drugs (XDR-TB)[1,2]. MDR-TB is characterized by MTB being resistant to the anti-tuberculosis minimal rifampicin (RIF) and isoniazid (INH) drugs. While XDR-TB becomes MTB with types that are resistant to INH and RIF and has resistance to one of the 2-line antibiotics of fluoroquinolones (FQs) and injection drugs with the types of amikacin (AMK) and kanamycin (KAN).Pre-XDR-TB is a disease caused by MTB strain that resists INH and RIF and either a fluoroquinolone or a second-line injectable drug, but not both[3,17].

Anti-tuberculosis drugs used as second-line drugs are Fluoroquinolones (FQs) groups ofloxacin (OFX)[4,5]. DNAgyrase is the main target of FQ in MTB. The *gyrA* and gyrB are mutation codes in quinolone-resistant determinants (QDRDs) associated with FQs resistance[6]. MTB bacteria are deactivated by inhibiting DNA replication and binding to DNA *gyrA*se[1,8,7,11]. Mutations in clinical MTB isolates with FQ resistance occur in the *gyrA* gene around 90%[7,19]. Whereas in the gyrB gene mutations occur lower or occur together with the *gyrA* gene[1,5,3].

The conventional method of testing the susceptibility of the MTB drug phenotypically to FQ takes several weeks to complete. Molecular diagnostic tests for the rapid detection of FQ resistance are urgently needed. In this study, we identified the association the result between the phenotype test and genotype test for MTB clinical isolates ofloxacin resistance.

II. Material And Methods

Isolates and phenotypic characterization

All of 42 MTB clinical isolates were collected from MDR-TB Laboratorium Haji Adam Malik Hospital in North Sumatera from January 2018 until June 2018. OFX resistance in all isolates was tested by the MGIT 960 method. The bacterium *Mycobacterium tuberculosis* H37Rv was used as a reference bacteria in this

study. This study was supported by The Health Research Ethical Committee of North Sumatera University / Haji Adam Malik Hospital.

DNA isolation

DNA isolation used to freeze-thaw cycling, a loopful of the MTB isolates from Lowenstein-Jensen medium was transferred to an mc-Cartney bottle that contained 1,5 distillate water then mixed by the vortex. Transfer the liquid to a 1,5 ml tube. Heat at 90°C for 10 minutes and then freeze in -20°C for 10 minutes until 6 cycles. The tube was then centrifuged at 13000 g for 5 minutes. The supernatant was transferred to a fresh 1,5ml tube and stored at -20°C until use.

PCR amplification of *gyrA* and gyrB genes

The *gyrA* gene amplification was amplified with the use of the *gyrA*F 5'CAG-CTA-CAT-CGA-CTA-TGC-GA3 primer and the *gyrA*R 5'GGG-CTT-CGG-TGT-ACC-TCA3 'primer. GyrB was amplified with the use of *gyrB*F 5'CGT-AAG-GCA-GAG-TTG-GT3 'primer and *gyrB*R 5'ATC-TTG-TGG-TGG-TAG-CGC-AGC-TT3' primer. The size of the amplified fragments was 320bp in both PCR products[8].

Interpretation of PCR products

The appearance of a band in 320 bp product amplification indicates the wild type and no mutations in the *gyrA* and *gyrB* genes. Missing fragments indicate mutations in the *gyrA* and gyrB genes. In this study, The DNA ladder used is 100 bp to analyze the band size[9,10].

III. Results

Phenotype test for clinical isolates *Mycobacterium tuberculosis*

The clinical isolate profiles of patients used in this study by sex consisted of 28/42 (66%) men and 14/42 (34%) women. Whereas based on age consisted of children 1/42 (2.3%), adolescents 7/42 (16.6%), adults 15/42 (35.7%) and elderly 9/42 (21.4%) . Among the 42 MTB isolates, 12(28,5%), 41(97,62%), 42(100%), 19(45,2%), 5(11,9%), 10(23,8%), and 42(100%) were resistant to streptomycin, isoniazid, rifampicin, ethambutol, amikacin, kanamycin, and ofloxacin by the conventional method on MGIT 960 liquid medium (table 1). Among 42 clinical isolates, the TB-RR, MDR-TB, Pre-XDR-TB and XDR-TB were 42/42 (100%), 41/42 (97,62%), 31/42 (73,90%), and 11/42 (26,1%). Not all TB-RR results from TCM examination for the diagnosis of MDR-TB are accompanied by isoniazid resistance from the phenotypic DST test results.

Table 1. Resistance pattern to the first line and second line of clinical isolates *Mycobacterium tuberculosis*

No	Type of resistance	Total isolates n (%)
1	Total isolates tested	42 (100%
2	Resistance to STR	12 (28,5%)
3	Resistance to INH	41 (97,62%)
4	Resistance to EMB	19 (45,2%)
5	Resistance to RIF	42 (100%)
6	Resistance to RIF+INH	41 (97,2%)
7	Resistance to RIF+INH+STR	12 (28,5%)
8	Resistance to RIF+INH+STR+EMB	9 (21,42%)
9	Resistance to AMK	5 (11,9%)
10	Resistance to KAN	10 (23,8%)
11	Resistance to OFX	42 (100%)
12	Total TB-RR	42 (100%)
13	Total TB-RR + Resistance OFX	42 (100%)
14	Total TB-RR + Resistance OFX + AMK	1 (2,38%)
15	Total TB-RR + Resistance OFX + KAN	5 (11,9%)
16	Total TB-RR + Resistance OFX + AMK + KAN	4 (9,52%)
17	Total MDR	41 (97,2%)
18	Total pre-XDR-TB	31 (73,8%)
19	Total XDR-TB	11 (26,19%)
20	Total resistance to all drugs	2 (4,76%)

Table 2. Profiles of clinical isolates population and comparison of TB-RR, MDR-TB, XDR-TB, and Pre-XDR-TB

Characteristics	Total(n=42)	TB-RR	MDR-TB	pre-XDR-TB	XDR-TB
Age-group					
children	1 (2,3%)	1 (2,3%)	1 (2,4%)	1 (3,2%)	
adults	38 (90.47%)	38 (90,4%)	37 (90,2%)	29 (93,5%)	9 (81,8%)
middle-aged	3 (7.14%)	3 (7,1%)	3 (7,3%)	1 (3,2%)	2 (18,1%)
Gender					
male	28 (66,6%)	28 (66,6%)	27 (64,2%)	20 (65,4%)	8 (72,7%)
female	14 (33,3%)	14 (33,3%)	14 (33,3%)	11 (35,4%)	3 (27,2%)

Genotype test for clinical isolates *Mycobacterium tuberculosis*

All 42 sputum samples were found to be TB-RR through genotype testing with gene Xpert (table 1). All *Mycobacterium tuberculosis* samples were positive and resistant to rifampicin drugs. GeneXpert only targets the *rpoB* gene hotspot region in *Mycobacteriumtuberculosis*. Therefore, it is necessary to increase the diagnostic capacity by genotyping for ofloxacin resistance using specific primers in the *gyrA* and *gyrB* genes.

Among the 42 MTB isolates, 37/42 (88,09%) isolates had mutations in *the gyrA* gene and 5/42 (11,90%) isolates were showed no mutation in *the gyrA* gene. The *gyrB* gene was shown no mutation in MTB clinical isolates. The results of mutations in *gyrA* was determined by polymerase chain reaction (PCR) for the 42 MTB ofloxacin resistant isolates.

Table 3. Percentage result of genotypic test *Mycobacterium tuberculosis* isolates

Mycobacterium tuberculosis	**% *gyrA* gene N=42**	**% *gyrB* gene n=42**
With mutations	88,1 %	0%
Without mutations	11,9%	100%
Total	100%	100%

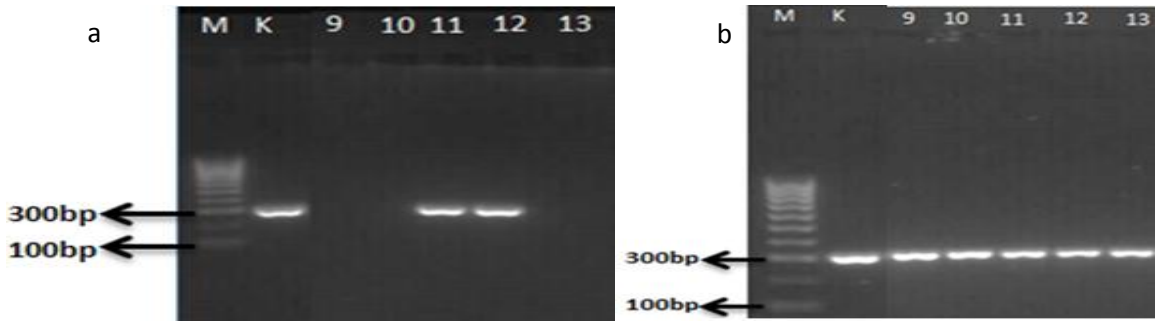

Figure 1. Agarose gel electrophoresis of PCR-assay for identification of (a) *gyrA* gene, (b) *gyrB* gene. M: 100bp ladder; K: Positive control (H37Rv); 9,10,11,12,13: isolates number.

As shown in figure 1, mutations in the *gyrA* gene isolate numbers 9,10 and 13 are indicated by the missing of fragments in agarose gel image of PCR products, while isolates numbers 11 and 12 can be seen that there is no mutation in the *gyrA* gene (a). In *the gyrB* gene, no mutation occurred at MTB isolates (b), which is marked by the band visualized in the agarose gel image of PCR products

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IV. Discussion

In this study, we have identified *gyrA* and *gyrB* genes from ofloxacin resistant MTB clinical isolates. Our study showed the mutations observed in *gyrA* QRDRs were 37 of 42 (88.1%) isolates had mutations and no mutations in 5 of 42 (11.9%) isolates in *gyrA.*

PCR detection results of the gyrB gene in all clinical MTB isolates that are resistant to OFX did not show mutations. Based on previous research, the*gyrA* gene mutation in MTB clinical isolates has been analyzed[11]. QRDR is known as the *gyrA* short mutation region, which is associated with the occurrence of FQs resistance in MTB[3]. Many mutation frequency studies have focused on the *gyrA* and gyrB genes mutations for FQs-resistant MTB isolates[11].

Cross-resistance to fluoroquinolones is a concern because they have different ways of working from classic first-line anti-TB drugs. Ofloxacin drug use has been widely used in the case of other infectious diseases. This drug is also freely used in several developed and developing countries. It has spurred increased FQs resistance in the treatment of TB patients[12].

Some reports showed that the most (probably 55%–90%) of FQs-resistant MTBhas a mutation in the *gyrA* gene in QRDR[11]and the *gyrB* gene has a small number mutation in FQs-resistant MTB[13,14]. This is consistent with research conducted by Chen et.al in 2012 in China that fluoroquinolone resistance to *Mycobacterium tuberculosis* is largely due to a *gyrA* gene mutation, whereas the *gyrB* gene mutation is rarely found[4]. This is because of 61 ofloxacin-resistant clinical isolates 88.5% had a *gyrA* gene mutation while no mutations were found in the *gyrB* gene in the study. Zhang et.al(2014) also mentioned that the *gyrB* gene mutation in the clinical isolate of *Mycobacterium tuberculosis* was only 2.9% of the total sample of 138 fluoroquinolone-resistant[15]. Saudani et.al (2010) in Tunisia found no mutation in the clinical isolate of *Mycobacterium tuberculosis* in the *gyrB* gene[16]. Research Nosova et.al (2013) in Moscow showed that only 5.3% of isolates found mutations in the gyrB gene while 94.7% found mutations in the *gyrA* gene[17]. Mutation in

the clinical isolate of *Mycobacterium tuberculosis* in the *gyrB* is alsoassociated with low-frequency OFX resistance.

V. Conclusions

The results of 42 MTB isolates can be concluded that overall 42/42 (100%) isolates were resistant to rifampicin and ofloxacin by phenotype method. In the genotyping method, mutations in the *gyrA* gene were found to be 37/42 (88.09%) isolates, while the number of sample mutations in the *gyrA* gene was 5/42 (11.90%). Meanwhile, in the *gyrB* gene, there are no mutations. This study also showed discordance result in 5 isolates, which in the phenotype test all isolates were showed resistant ofloxacin but in genotype test, that isolates were showed no mutation in the *gyrA* and the *gyrB* gene.

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