PROFILE OF gyrA AND gyrB GENE MUTATION IN CLINICAL ISOLATE OF CIPROFLOXACIN RESISTANT *Pseudomonas aeruginosa*

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ABSTRAK

*Infeksi nosokomial dapat meningkatkan morbiditas dan mortalitas, serta biaya pengobatan karena perawatan di rumah sakit menjadi lebih lama. Salah satu bakteri penyebab infeksi nosokomial terbanyak adalah Pseudomonas aeruginosa. Siprofloksasin merupakan anti Pseudomonas yang paling kuat, dimana memiliki 4-8 kali lebih aktif dibandingkan Levofloksasin terhadap Pseudomonas aeruginosa. Resistensi terhadap antibiotika siprofloksasin dapat disebabkan oleh mutasi pada DNA girase dan topoisomerase IV. Mutasi DNA girase pada bakteri Pseudomonas aeruginosa dapat terjadi pada gen gyrA dan gyrB. Penelitian ini bertujuan untuk melihat mutasi gen gyrA dan gyrB pada isolate klinik Pseudomonas aeruginosa yang resisten terhadap siprofloksasin dengan menggunakan QiaQuick PCR column (QIAGEN, Valecia, CA) dan Genetic Analyzer ABI Prisma 310. Sebanyak 5 isolate Pseudomonas aeruginosa menunjukkan fenotip yang resisten terhadap siprofloksasin dan mengalami mutasi pada gen gyrA dengan perubahan asam amino Thr83 menjadi Ile sebesar 100%. Hal ini menunjukkan bahwa mutasi pada gen gyrA sangat berpengaruh terhadap resistensi bakteri Pseudomonas aeruginosa terhadap siprofloksasin.**(FMI 2014;50:81-85)***

Kata kunci:*Pseudomonas aeruginosa, gen gyrA, gen gyrB, mutasi, siprofloksasin*

ABSTRACT

*Nosocomial infections can increase morbidity, mortality, and also the cost of treatment because hospital care becomes longer. One of the most bacteria causing nosocomial infections is Pseudomonas aeruginosa. Ciprofloxacin is the most powerful antipseudomonal, which has 4-8 times more active than levofloxacin, against Pseudomonas aeruginosa. Ciprofloxacin antibiotic resistance can be caused by mutations in DNA gyrase and topoisomerase IV. DNA gyrase mutations in bacteria Pseudomonas aeruginosa can occur in genes gyrA and gyrB. This study aims to see gyrA and gyrB gene mutations in clinical isolates of Pseudomonas aeruginosa were resistant to ciprofloxacin by using PCR QiaQuick column (QIAGEN, Valecia, CA) and the ABI Prism 310 Genetic Analyzer. All 5 Pseudomonas aeruginosa isolates of showed phenotypes that were resistant to ciprofloxacin and gyrA gene mutation in the amino acid change at Thr83 to be Ile at 100%. This suggests that mutations in the gyrA gene affects the occurrence of bacterial resistance of Pseudomonas aeruginosa to ciprofloxacin. **(FMI 2014;50:81-85)***

Keywords:*Pseudomonas aeruginosa, gyrA gene, gyrB gene, mutation, ciprofloxacin*

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INTRODUCTION

Nosocomial infections generally occur in the urinary tract (42 %), pneumonia (21 %), surgical wound (16%), and sepsis (8%). One of the most bacteria causing nosocomial infections is *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is able to invade the tissues of the body through the production of enzymes and toxins (Mesaros et al 2007). Floroquinolon class of antibiotic widely used for the treatment of *Pseudomonas aeruginosa* infections. Ciprofloxacin is the most powerful anti Pseudomonas, which has 4-8 times more active against *Pseudomonas aeruginosa* compared with levofloxacin. Ciprofloxacin antibiotic resistance can be caused by mutations in DNA gyrase and topoisomerase IV which causes changes in the binding sites of target molecules work the antibiotic ciprofloxacin (Owens & Ambrose 2001).

DNA gyrase mutations in bacteria *Pseudomonas aeruginosa* can occur in genes gyrA and gyrB. Mutations in the gyrA gene is often the case that an amino acid substitution at Thr83 to be Ile with base change at position ATC to be ACC andAsp87 to be Asn with base change at position AAC to be GAC. Mutation in the gyrB gene is an amino acid substitution at Ser466 to be Phe with base change at position TCC to be TTC and Glu468 to be Asp with base change at position GAG to be GAC. Although mutations in the gyrB subunit and pare generally rare, but mutations in gyrB

and pare can subunits associated with an increase in quinolone resistance (Akasaka et al 2001, Lee et al 2005, Mouneimné et al 1999, Wydmuch et al 2005).The purpose of this study is to determine the genetic mutation profile of gyrA and gyrB gene regions at *Pseudomonas aeruginosa* isolates were resistant to ciprofloxacin by using PCR and sequencing.

MATERIALS AND METHODS

The sensitivity test of *Pseudomonas aeruginosa* clinical isolates to ciprofloxacin was performed using the BD Phoenix automated microbiology. Procedure to determine the profile of gyrA and gyrB genes mutations from *Pseudomonas aeruginosa*clinical isolates were extraction of DNA using the Extract-N ampiTM Blood PCR Kit, then followed by Polymerase Chain Reaction (PCR) for genes gyrA and gyrB genes. Primers used were F (5'-GTCCTATCTCGACTACGCGAT-3') at position 320-341 and R (5'-GTCGACGGTTTCCTT TTCCAG-3') at position 676-697 for genes gyrA and F (5'-GCGCGAGATGACCCGCCGCA-3') in position from 1170 to 1189 and R (5'-CTGGCGGAAGAAGGT CAACA-3') position from 1918 to 1995 for the gyrB gene.

The amplification was by means a thermal cycler machine BioRadicycler. Then the process of electrophoresis was performed on 2% agarose in Tris Borate solution. Labeling was done using PCR QiaQuick column (QIAGEN, Valecia, CA) and DNA sequencing using the ABI Prism 310 Genetic Analyzer.

RESULTS

All five *Pseudomonas aeruginosa* isolates showed ciprofloxacin resistant (resistance with MIC score >2 µg/ml). Obtained from electrophoresis process, gyrA gene has 377 bp, while gyrB gene has 500 bp single band. Sequencing result of gyrA gene showed amino acid change (missense mutation) in isolate number 1,2,3,4,5 (Thr83 to be Ile and Gly105 to be Ala), isolate number 1,2,3,5 (Gln106 to be Arg), isolate number 4 (Gln53 to be Ser), isolate number 2,3,4 (Ala136 to be Gly), while there are amino acid changes in gyrB gene isolate number 4 (Asp394 to be Tyr). Silent mutation, which did not change amino acid form, was found in gyrA gene isolate number 3 (Ala51) and isolate number 4,5 (Gly56) and in gyrB gene, isolate number 4 (Arg274, Glu275,346, Ala277,348, Lys298).

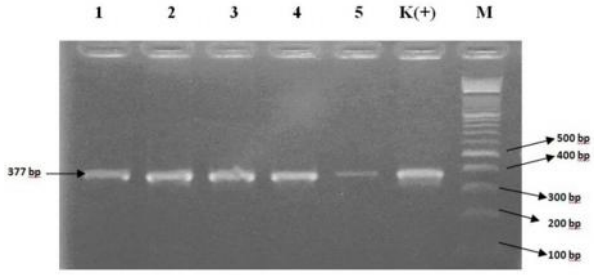

Figure 1. Electrophoresis results gyrA gene

Information:
K (+): Positive control (pure strains *Pseudomonas aeruginosa*)
M:Ladder Markers with a distance of 100 bp
1,2,3,4,5:Clinical isolates number
bp: base pair
377 bp:PCR optimization results obtained single band identical with control of 377 bp.

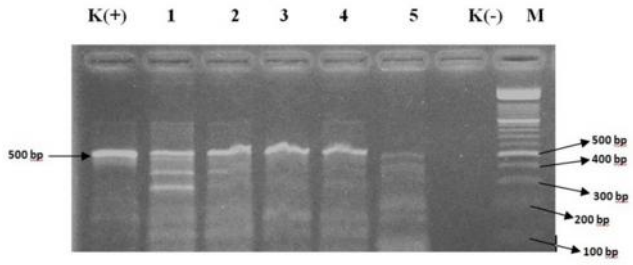

Figure 2. Electrophoresis results gyrB gene

Information:
K (+): Positive control (pure strains *Pseudomonas aeruginosa*)
K (-):Negative control (aquadest)
M:Ladder Markers with a distance of 100 bp
1,2,3,4,5:Clinical isolates number
bp: base pair
500 bp:PCR optimization results obtained single band identical with control of 500 bp.

DISCUSSION

In this study, five clinical isolates of testing resistance to the antibiotic ciprofloxacin using the BD Phoenix automated microbiology system which produces an interval of values. Minimum Inhibitory Concentration (MIC). This method has a high degree of practicality because it can detect antimicrobial resistance of specimens in relatively large amount, but it has the disadvantage that can't demonstrate the type of mutation relationship with MIC. Combination with conventional methods used such an indispensable test dilution method or the disc diffusion test.

In this research, the process of DNA replication (amplification) in the study sample by PCR was followed by electrophoresis of the gyrA and gyrB genes in order to obtain all the samples showed a single band with a ribbon specific to gyrA gene of 377 bp and 500 bp for gyrB gene. GyrA and gyrB genes are 2 sub- units of DNA gyrase enzyme which plays an important role in the process of replication, transcription and recombination of DNA (Drlica et al 2008, Bradford & Dean 2008). Both sub-units are part of a quinolone Resistance Determining Regions (QRDR) in addition to genes parC and parE which is a sub unit of the enzyme topoisomerase IV. In gram negative mutations in the DNA gyrase occurs is that the primary mutation, in topoisomerase IV is an additional mutation. The occurrence of mutations in gyrA and gyrB genes cause changes in the DNA gyrase enzyme which can lead to ciprofloxacin can't work on the target and there is resistance to ciprofloxacin.

Figure 3. The results of cluster alignment amino acid sample gyrA gene of *Pseudomonas aeruginosa* isolates and standard (Gen Bank Acession Number L29417).

Information:
L29417:Gen Bank Acession Number for standard
gyrA-6: Pure Strains bacteria *Pseudomonas aeruginosa* as research control.
gyrA-1 to gyrA-5: Clinical Isolates *Pseudomonas aeruginosa* as research sample
1-109: Length of an amino acid, compared to determine the presence or absence of amino acid changes (mutations)

Figure 4. The results of cluster alignment amino acid sample gyrB gene of *Pseudomonas aeruginosa* isolates and standard (Gen Bank Acession Number AB005881).

Information:
AB005881:Gen Bank Acession Number for standard
6-F-AA: Pure Strains bacteria *Pseudomonas aeruginosa* as research control
1- F-AA s/d 5-F-AA: Clinical Isolates *Pseudomonas aeruginosa* as research sample
1-142: Length of compared amino acid

Table 1. Quinolon Resistance Determining Regions

QRDRs*	Isolate Number	Codon Position	Change in Nucleotide Base	Amino Acid Change	Percentage
	3	51	GCC→GCG	Alanine	20%
	4	53	GGC→AGC	Glutamine →Serine	20%
	4,5	56	GGC→GGG	Glycine	40%
gyrA	1,2,3,4,5	83	ACC→ATC	Threonine→Isoleusin	100%
	1,2,3,4,5	105	GGC→GCC	Glycine→Alanine	100%
	1,2,3,5	106	CAG→CGG	Glutamine→Arginin	80%
	2,3,4	136	GCG→GGG	Alanine→Glycine	60%
		274	CGT→CGC	Arginine	20%
		275	GAA→GAG	Glutamic Acid	20%
		277	GCG→GCA	Alanine	20%
gyrB	4	298	AAA→AAG	Lysine	20%
		346	GAG→GAA	Glutamic Acid	20%
		348	GCA→GCG	Alanine	20%
		394	GAC→TAC	Aspartic Acid→Thyrosine	20%

Information:
*QRDRs:Quinolon Resistance Determining Regions
missense mutation:gyrA gene atcodon 53, 83, 105, 106 dan 136
silent mutation: gyrA gene atcodon 51 and 56, gyrB gene at codon 274, 275, 277, 298, 346 and 348.

Mutations that occur in the gyrA subunit genes generally occur in amino acids at positions 83 and 87. It is 83 amino acids in the normal state is a Serine or Threonine can be changed to leucine, tryptophan, Alanine, isoleucine, phenylalanine, or arginine; while the 87 amino acids that are normally in the form of aspartic acid can be changed into asparagine, valine, glycine, tyrosine, alanine, histidine, valine, or lysine. For the mutation occurs in the gene gyrB amino acid substitution at position 466 is a Serine into phenylalanine later substitution at position 468 the amino acid glutamic acid into aspartic acid (Lee et al 2005, Wydmuch et al 2005, Akasaka et al 2001, Mouneimné et al 1999).

There are five clinical isolates of *Pseudomonas aeruginosa* showed phenotypes that were resistant to ciprofloxacin and has undergone a missense mutation, which for the gyrA gene that Gln53 to be Ser (20%), Thr83 to be Ile (100%), Gly105 to be Ala (100%), Gln106 to be Arg (80%), Ala136 to be Gly (60%). The results of the study of mutations in the gyrA gene showed a considerable influence on the occurrence of bacterial resistance of *Pseudomonas aeruginosa* to ciprofloxacin. GyrB gene mutations in gram-negative bacteria occur at codon position number 406-495 range. In the study to the *E. coli* bacteria, there were amino acid changes of aspartic acid into asparagine codon at position number 426 and lysine to glutamic acid codon at position number 447. Mutations at codon 426 occurred in all samples at codon 447, while an increase in the occurrence of resistance but only in the antibiotic nalidixic acid, whereas for the other quinolone antibiotics are still sensitive. In this research mutations to genes gyrB among other sample number 4 amino acid change of aspartic acid to tyrosine with base change GAC to be TAC codon at position number 394 (20%).

CONCLUSION

There are five *Pseudomonas aeruginosa* isolates of showed phenotypes that were resistant to ciprofloxacin and has undergone a missense mutation, which for the gyrA gene (Gln53 to be Ser (20%), Thr83 to be Ile (100%), Gly105 to be Ala (100%), Gln106 to be Arg (80%), Ala136 to be Gly (60%)) and for the gyrB gene (Asp394 to be Tyr (20%)). Mutations in the gyrA gene affect the occurrence of bacterial resistance of *Pseudomonas aeruginosa* to ciprofloxacin.

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