THE PROFILE OF GENETIC MUTATION OF THE gyrB GENE ON CLINICAL ISOLATION OF *Acinetobacter baumannii* RESISTANT TO CIPROFLOXACIN

Marich Amilia Rizka[1], Didik Hasmono[1], Kuntaman[2]
[1]Department of Clinical Pharmacy, Faculty of Pharmacy, Universitas Airlangga
[2]Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga

ABSTRAK

Infeksi nosokomial didefinisikan sebagai suatu infeksi yang didapat di rumah sakit atau fasilitas kesehatan lainnya oleh penderita. Salah satu bakteri penyebabnya adalah Acinetobacter baumannii. Terapi infeksi nosokomial yang disebabkan Acinetobacter baumannii antara lain menggunakan antibiotik golongan florokuinolon (ciprofloxacin khususnya). Mekanisme resistensi Acinetobacter baumannii terhadap ciprofloxacin umumnya dikarenakan mutasi pada gen penyandi enzim DNA girase (gen gyrA dan gen gyrB). Penelitian ini bertujuan untuk melihat adanya mutasi gen gyrB pada isolat klinik Acinetobacter baumannii yang resisten terhadap ciprofloxacin dengan menggunakan QiaQuick PCR column (QIAGEN, Valecia, CA) dan Genetic Analyzer ABI Prisma 310.Sebanyak 7 isolat klinik Acinetobacter baumannii menunjukkan fenotip yang resisten terhadap ciprofloxacin. Berdasarkan hasil sekuensing terhadap gen gyrB menunjukkan perubahan asam amino pada isolat nomor 2 (Glu479 → Asp), nomor 4 (Cys423 → Ser dan Glu479 → Asp), nomor 5 (Cys423 → Ser), nomor 6 (Glu479 → Asp), dan nomor 7 (Leu420 → Gln; Cys423 → Ser; Leu433 → His; dan Glu479 → Asp), dengan jenis mutasi missense mutation, suatu mutasi yang disertai dengan perubahan asam amino. Resistensi Acinetobacter baumannii terhadap ciprofloxacin pada penelitian ini diakibatkan oleh mutasi pada gen gyrB, namun dilihat dari besaran persentase mutasinya, peranannya untuk pola resistensi tidak terlalu dominan. ***(FMI 2014;50:215-218)***

Kata Kunci*: Acinetobacter baummannii, gen gyrB, mutasi, ciprofloxacin*

ABSTRACT

Nosocomial infection is defined as an infection acquired from a hospital or other health facilities by the patient. One of the causing bacteria is Acinetobacter baumannii. Nosocomial infection therapy caused by said bacteria includes: Using the floroquinolone antibiotics (especially ciprofloxacin). The resistance is caused by a mutation on the gene that codes the DNA gyrase enzyme (gyrA and gyrB gene). This research was performed to examine the gyrB gene mutation on the clinical isolate, Acinetobacter baumannii that is resistant to ciprofloxacin by using QiaQuick PCR column (QIAGEN, Valecia, CA) dan Genetic Analyzer ABI Prisma 310. Seven clinical isolates show a resistance against ciprofloxacin phenotype. Base on the sequencing result of the gyrB gene, there is an amino acid change on isolate: number 2 (Glu479 → Asp), number 4 (Cys423 → Ser and Glu479 → Asp), number 5 (Cys423 → Ser), number 6 (Glu479 → Asp), and number 7 (Leu420 → Gln; Cys423 → Ser; Leu433 → His; and Glu479 → Asp), with a missense mutation, a mutation associated with amino acid change. The resistance in this research is caused by the gyrB gene, however, examined through the mutation's percentage, its role on the resistance is not highly dominant. ***(FMI 2014;50:215-218)***

Keywords: *Acinetobacter baummannii, gyrB gene, mutation, ciprofloxacin*

Correspondence: Marich Amilia Rizka, Department of Clinical Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Dharmawangsa Dalam Surabaya 60286 – Indonesia, http://www.ff.unair.ac.id, e-mail address: marichamiliarizka@gmail.com

INTRODUCTION

Nosocomial Infection that often happens is a surgical wound infection, urinary tract infection, and lower respiratory tract infection. One of the bacteria that causes nosocomial infection is *Acinetobacter baumannii*, which is a pathogenic bacteria that causes a nosocomial infection with a high morbidity and mortality. One of the nosocomial infections, which is caused by *Acinetobacter baumanni*, therapies uses antibiotics like floroquinolone. Ciprofloxacin is the most-used due to its high activity when used against gram-negative bacteria (Torres et al 2009, Deris et al 2009).

Resistance against antibiotics ciprofloxacin can be caused by a mutation on the gyrase DNA (gyrA and gyrB) and topoisomerase IV (parC and pare). *Acinetobacter baumannii*'s resistance mechanism against ciprofloxacin is generally caused by a mutation on the gene that encodes the DNA gyrase enzyme (gene gyrA and gene gyrB). Mutation on gene gyrB, that occurs mainly on the codon 426 and 447 426th and 447th codon, clinically it is not really significant and rarely found if compared to the mutation on the gyrA

gene. Even though the mutation on the gyrB gene can e correlated with the increase of quinolone resistance (De la Fuente et al 2007). Based on the background above, we are planning to perform a research to find out whether there is a genetic mutation in the gyrB gene area against the clinical isolate, *Acinetobacter baumannii*, which has been proven to be resistant to ciprofloxacin.

MATERIALS AND METHODS

The sensitivity test of *Acinetobacter baumannii* clinical isolates to ciprofloxacin was performed using the BD Phoenix automated microbiology. Procedure to determine the profile gyrB genes mutations from *Acinetobacter baumannii* 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 for the gyrB gene were F (5'-GTGAAATGACGCGTCG TAAG-3') and R (5'-CGAATGTGTGAACCATCG AC-3'). The amplification was done by means a thermal cycler machine BioRadicycler. Then the process of electrophoresis was performed on 2% agarose in Tris Borate solution. Labeling was using PCR QiaQuick column (QIAGEN, Valecia, CA) and DNA sequencing was using the ABI Prism 310 Genetic Analyzer (Perkin Elmer).

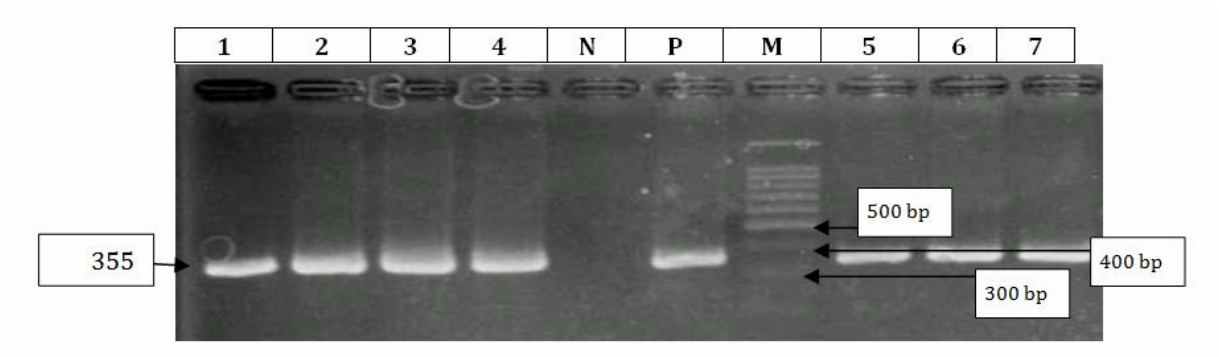
RESULT

Seven clinical isolates of *Acinetobacter baumannii* show resistant phenotype against ciprofloxacin. From the amplification result using the primary gyrB gene and electrophoresis, annealing optimal temperature is acquired on 60°C and 355 bp (base pair) of specific band or single tape. Based on the sequencing result of the gyrB gene, it shows a change of amino acid of isolate number 2 (Glu479 → Asp), number 4 (Cys423 → Ser and Glu479 → Asp), number 5 (Cys423 → Ser), number 6 (Glu479 → Asp), and number 7 (Leu420 → Gln; Cys423 → Ser; Leu433 → His; and Glu479 → Asp) with a missense mutation, a mutation associated with amino acid change.

DISCUSSION

In this research, sequencing to determine the mutation profile of gyrB gene that affects the resistance of the bacteria *Acinetobacter baumannii* was performed after performing ciprofloxacin resistance test by using Minimum Inhibitory Concentration (MIC) method, the Automatic Microbiology System Method of BD Phoenix. This method is highly practical due to the fact that it can detect resistance from many antimicrobial specimens. The disadvantage of determining the MIC using this system is that it cannot present the correlation of mutation type with MIC.

In this research, the process of DNA replication is performed (amplification) with PCR method. The process of amplification using primary gyrB referring to the research by De la Fuente et al (2007). The process of amplification by using the primary reference to determine that the area that is going to be amplified is sequence or consecutive DNA of gyrB from *Acinetobacter baumannii*. After the process of amplification, electrophoresis is perfumed and a single band of 355 bp is acquired.



Lane 1,2,3,4,5,6,7 : Sample of DNA gyrase B *Acinetobacter baumanii*
Lane P : Positive Control using pure strained *Acinetobacter baumanii* (EU73)
Lane N : Negative Control
Lane M : Ladder mark 100 bp (100 base pair)

Figure 1. gyrB gene electrophoresis result

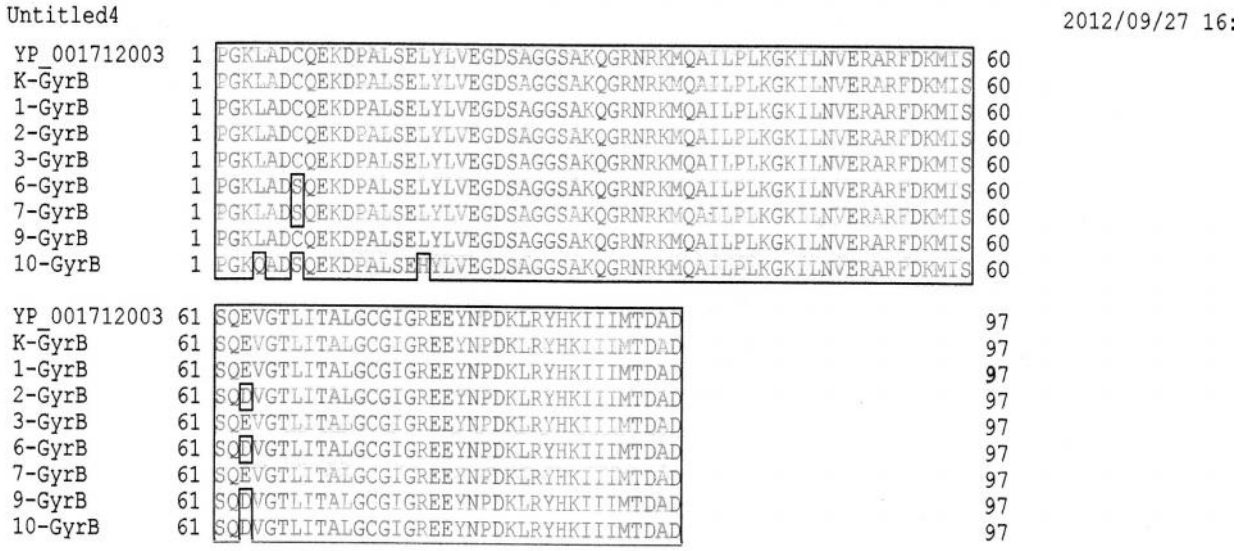


Figure 2. Result of cluster alignment research sample of *Acinetobacter baumannii* gyrB with control and standard (Gen Acession YP_001712003)

Table 1. Mutation and Location of *Acinetobacter baumannii* isolate mutation of gen gyrB

Isolate Number	Codon Position	Codon Change	Amino Acid Change	Mutation Percentage
7	420	TTG→CAG	Leu→Gln	14.29 %
4, 5, 7	423	TGT→ AGT	Cys→Ser	42.86 %
7	433	TTA→CAC	Leu→His	14.29 %
2, 4, 6, 7	479	GAA→GAT/GAC	Glu→Asp	57.14 %

In this research, 4 points of different codon location was found. In the area, mutation that occurred created a change of amino acid from the isolate. The change of amino acid that occurred includes Leusin into Glutamin on codon 420 (Leu420 → Gln), Sistein into Serin on codon 423 (Cys423 → Ser), Leusin intoHistidin on codon 433 (Leu433 → His), dan Glutamic Acidintoi Aspartic Acid on codon 479 (Glu479 → Asp). The result produces new knowledge regarding the mutation of gyrB gene on the *Acinetobacter baumannii* bacteria resistant to ciprofloxacin. According to the research of De la Fuente et al (2007), mutation on gyrB gene is rarely found.

The mechanism of ciprofloxacin antibiotic is by inhibiting the synthesis of bacteria DNA. This inhibition occurred due to the interaction between drugs with complex that is formed by DNA and enzymes that are work targets, gyrase DNA (gyrA and gyrB) and topoisomerase IV, therefore, after a mutation of the enzyme occurs, the ciprofloxacin antibiotic is no longer usable even if the dose is increased. Based on the result, mutation of the gyrB gene is associated with amino acid change, the change may produce a new kind of phenotype, a resistant bacteria. This shows the role of the mutation of the gyrB gene on the process of *Acinetobacter baumannii*'s resistance towards Ciprofloxacin

CONCLUSION

The *Acinetobacter baumannii* isolate is resistant to cipfofloxacin that have undergone a mutation of gyrB on codon: 420 (14.29%), 423 (42.86 %), 433 (14.29 %), and 479 (57.14 %) with a missense mutation (the change of nucletiotide arrangement that causes an amino acid change) as the type of mutation. The amino acid change is from Leusin into Glutamin, Cystein into serine, leusine into histidine, glutamic acid into aspartic acid. The resistance against ciprofloxacin in this research is caused by a mutation on the gyrB gene.

REFERENCES

Deris ZZ, Harun A, Shafei M, Rahman AR, Johari RM (2009). Outcome and appropriates of management of

mosocomial Acinetobacter bloodstream infections at a teaching hospital in northeastern Malasyia. Southeast Asian J Trop Med public Health 40, 140-147

De la Fuente CM, Dauros SP, Bello TH, Domínguez YM (2007). Mutations in gyrA and gyrB genes among strains of Gram-negative bacilli isolated from Chilean hospitals and their relation with resistance to fluoroquinolones. Rev Med Chil 135, 1103-1110

Torres A, Ewig S, Lode H, Carlet J, European HAP working group (2009). Defining, treating and preventing hospital acquired pneumonia: European perspective. Intensive Care Med 35, 9-29