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Research Article

Identification of gyrA Gene Resistance on Bacteria *Escherichia coli* and *Klebsiellapneumoniae* in Urinary Tract Infection Patients in Haji Adam Malik Center Hospital, Medan, Indonesia

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ABSTRACT

Objectives: To find out whether *Escherichia coli* and *Klebsiellapneumoniae* that cause urinary tract infection have ciprofloxacin resistant gyrA coding genes.

Interventions: The method used in this study is a cross sectional prospective design and carried out prospectively in urinary tract infection patients who have medical record data on the diagnosis urinary tract infection patients in the period February 2019 to January 2020 at the Haji Adam Malik General Hospital in Medan by takingurine from patients, then detected using Polymerase Chain Reaction (PCR) in the Integrated Laboratory of the Faculty of Medicine, Universitas Sumatera Utara.

Main outcomes measure: The resistance coding gene in *Escherichia coli*with the percentage of gyrA gene is 96,1% and *Klebsiellapneumoniae* has a low percentage where the expression of gyrA gene is 66,6%.

Conclusion: The distribution of the gyrA gene in *Escherichia coli* is higher than that of *Klebsiellapneumoniae.*

Keywords: gyrA gene, *Escherichia coli, Klebsiellapneumoniae*, urinary tract infection.

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INTRODUCTION

Urinary tract infections (UTIs) remain one of the most common infectious diseases in humans both in outpatients and inpatients worldwide[1]. Urinary tract infections (UTIs) is one of the most common infectious diseases, ranks second after respiratory infections[2]. Fluoroquinolones (FQs)havebeen the most commonly used antibiotics to treat UTIs caused by Gramnegative bacteria[3].

Basedon Microbiology culture data reports obtained from antimicrobial resistance in uropathogens and appropriateness of empirical treatment: a population-based surveillance study in Indonesia from April 2014 to May 2015, with a total of 3380 patients. Urinary tract infections was the highest caused by gram negative bacteria *Escherichia coli* as much as 17,01% and the high rate os resistance to Ciprofloxacin antibiotics is 48,3% -71,6% in patients with urinary tract infections, so it can be a clinical consideration for not using ciprofloxacin as therapy empirical[4].

The most common mechanism of high-level resistance is due to mutations within the *quinolone resistance determining regions* (QRDRs) of at least one of the genes that encode the primary and secondary targets of these drugs, the type II topoisomerases (gyrA, gyrB, parC and parE). In *Escherichia coli*, the most common mutation site in gyrA, both key residues for quinolone binding, and similar mutation frequencies are seen at equivalent positions for GyrA and ParC in other species[5].

The formation of quinolone–topoisomerase–DNA ternary complexes causes the DNA replication machinery to become arrested at blocked replication forks, resulting in an inhibition of DNA synthesis, which immediately leads to bacteriostasis (at low quinolone concentrations) and eventually to cell death (at lethal concentrations) [6].

Research in the Iran in a2018 showed prevalence of gyrA (QRDR) resistance genes in *Escherichia coli* (60.4%) and *Klebsiellapneumoniae* (6.8%)[7]. Research in the Indonesiain a 2017 showed prevalence of gyrA (QRDR) resistance gene in *Escherichia coli* was 94%[8].

This article discusses the results of identifying the gyrA gene mutation that causes urinary tract infection in the Central General Hospital of Haji Adam Malik, Medan, Indonesia.

METHODS

This study uses the Cross Sectional Prospective Design method conducted prospectively to determine and determine the mutation of the bacterial gene that causes resistance to fluoroquinolone in urinary tract infection patients who meet the criteria for the period February 2019 to January 2020 at the Haji Adam Malik General Hospital Medan,

The study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara/ Haji Adam Malik Hospital.

Population and Sample

The population in this study was suspected urinary tract infection Hospital of Haji Adam Malik Medan during the period from February 2019 to January 2020. Sampling as a research subject is carried out by using purposive sampling method. Sample selection is based on certain characteristics that are related to population characteristics that have been known previously. The sampling distribution curve will center on 10% of the population parameter and will have all the properties of the normal distribution[9]. The sample in this study amounted to 50 people based on the above theory.

Urine Sampling

Urine extraction was taken aseptic method the middle portion of the urine, removed 1/3 the first and last urin. Urine samples are put into an ice cooler and then directly taken to the Integrated Laboratory of the Faculty of Medicine, Universitas Sumatera Utara to be used as research material for the identification of gene mutations that occur in bacteria that cause urinary tract infection. If the sample has not been used, it should be stored at 2-8°C.

Urine Urinary Tract Infection Sample Extraction

The sample is stored in an incubator of 30°C for 20 minutes, then the sample is prepared for the DNA extraction process[10]. Urinary tract infections bacterial DNA extraction was carried out using Geneaid® Urine DNA Isolation Kit. The extraction results were tested for DNA purity by calculating ratios at wavelengths of 260 and 280 nm using a nanophotometer to obtain results of 1.8-2.0 which showed that bacterial DNA had high purity and could be amplified using PCR.

gyrA gene Amplification

Gene detection begins by making 25µl amplification consisting of 12,5 µl mastermix, 1 µl reverse primer, 1 µl forward primer, 8,5 µl free water nuclease and 2µl bacterial DNA used as a template inserted into a micro tube 0,2 µl[11,12]. Gene amplification was carried out by PCR in specific primers of gyrA with thermocycling conditions as in table 1 below.

Table: 1 Spesific Primary gyrA gene

Primer Name Primer Sequence (5' a 3')	Target Gene	Product Size (bp)	Thermocycling	
gyrA (F)			Denaturation 94°C (5 min) 1 Cycle	
ACG TAC TAG GCA ATG ACT GG	gyrA	264	Amplification Denaturation (1 min) 94° C Anneling (30 sec) 55° C Extension (1 min) 72° C	30 Cycle
gyrA (R)				
AGA AGT CGC CGT CGA TAG AA			Final Extension (5 min) 72° C 1 Cycle	

Detection of PCR Results with Agarosa Gel Electrophoresis

As much as 2% agarose in 130 mL trisacetate EDTA (TAE) was heated until dissolved, then the solution was allowed to stand until warm and added 1 µL of ethidium bromide (EtBr) then shaken until homogeneous then poured into a mold and allowed to stand for 30 minutes until it was completely frozen. A total of 5 µL of PCR and marker samples were putinto 2% agarose gel wells. The electrophoresis process was carried out with a potential difference of 100 V, 400 Ma for 60 minutes. Amplified DNA that has been electrophoresed is visualized using Gel Documentation. DNA bands will be seen and their size can be determined based on molecular size markers expressed by base pairs[13].

The collected data were analyzed descriptively, where positive DNA results were shown with an amplicon of 264 bp. The negative control (N) used is sterile aquadest. The marker (M) used is a DNA ladder of 100 bp.

RESULTS AND DISCUSSION

The results of this study showed that of 50 people diagnosed with urinary tract infection grouped by sex, namely women with urinary tract infection more than 30 people (60%) compared to men as many as 20 people (40%). Patients with the most urinary tract infection are patients over 40 years old (26%) with an average age of 44,30 years, followed by patients over 50 years old (24%), and the lowest age is 20-30 years (12%).

The results of research on 50 urine carried out by PCR method obtained 38 urine that expressed *Escherichia coli* (26 sample) and *Klebsiella pneumoniae* (12 sample). The

distribution of resistance genes in both bacteria can be seen in the table 2 and 3.

Table 2.gyrA Gene Distribution in *Escherichia coli*

Sample Number	Speciment	gyrA (%)
2	Urine	(+)
3	Urine	(+)
5	Urine	(+)
7	Urine	(+)
8	Urine	(+)
10	Urine	(+)
12	Urine	(+)
14	Urine	(+)
16	Urine	(+)
18	Urine	(+)
20	Urine	(+)
24	Urine	(+)
25	Urine	(+)
26	Urine	(+)
27	Urine	(+)
29	Urine	(+)
31	Urine	(+)
33	Urine	(-)
36	Urine	(+)
39	Urine	(+)
40	Urine	(+)
44	Urine	(+)
45	Urine	(+)
46	Urine	(+)
48	Urine	(+)
50	Urine	(+)
Total 26 Samples		**25 (96,1%)**

Table 3.gyrA Gene Distribution in *Klebsiella pneumoniae*

Sample Number	Speciment	gyrA (%)
4	Urine	(+)
9	Urine	(-)
11	Urine	(+)
17	Urine	(-)
23	Urine	(+)
28	Urine	(+)
30	Urine	(-)
32	Urine	(-)
37	Urine	(+)
38	Urine	(+)
43	Urine	(+)
48	Urine	(+)
Total 12 Samples		**8 (66,6%)**

The expression of gyrA is higher in *Escherichia coli* because of the high prevalence of gyrA gene in the antibiotic resistant *Escherichia coli*. FQ resistance is mostly attributed to mutations targeting the QRDRs of DNA gyrase and topoisomerase IV[14]. Two essential bacterial enzymes that modulate the chromosomal supercoiling required for critical nucleic acid processes. The acquisition of quinolone resistance is recognized to be multifactorial and complex. The main resistance mechanism consists of one or a combination of target-site gene mutations that alter the drug-binding affinity of target enzymes[15].*Escherichia coli* is a producer of gyrA and is a common type of gene. The results showed that *Escherichia coli* and *Klebsiella pneumoniae* with a high percentage of the presence of gyrA genes.

CONCLUSSION

The distribution of the gyrA gene in *Escherichia coli* is higher than that of *Klebsiella pneumoniae*.

CONFLICT OF INTEREST

All author have no to declare.

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