The Bacterial Susceptibility of Levofloxacin for Urinary Tract Infections Bacteria in Type 2 Diabetes Mellitus Patients

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Zulfikar Dwi Kiswahyu Nindo[1], Mahyarudin Mahyarudin[2*], Mardhia Mardhia[2], Andriani Andriani[2]

[1]Medical Study Program, Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia
[2]Department of Microbiology, Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia
[3]Department of Biochemistry and Biomolecular, Faculty of Medicine, Universitas Tanjungpura, Pontianak, Indonesia

*Corresponding author: Mahyarudin
Email: mahyarudin@medical.untan.ac.id

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Abstract

Background: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, and can be associated with long term organ damage, organ dysfunction, and multiple organ failure. Type 2 DM causes high glucose levels in urine and suppression of the immune system, as in result it becomes a predisposing factor for urinary tract infections (UTIs). The antibiotic therapies that can be given in UTIs is levofloxacin which is effective against many types of bacteria that cause UTIs. This study aimed to determine the pattern of levofloxacin sensitivity against the bacteria that cause UTIs patients with type 2 DM.

Methods: The study used a descriptive method with a total sample of 22 bacterial isolates that had been isolated from UTIs patients with type 2 DM. The bacterial isolates were collections of the Microbiology Laboratory, Faculty of Medicine, Tanjungpura University. The study was conducted from June to October 2019. The antibiotic sensitivity test used the disc diffusion method and interpretation based on the Clinical & Laboratory Standards Institute (CLSI).

Results: levofloxacin was sensitive to UTIs bacteria by 95,45% with a total of 21 isolates from 22 isolates. The levofloxacin sensitivity pattern which was sensitive to types of bacteria such as Escherichia coli (100%), Pseudomonas aeruginosa (100%), Enterobacter aerogenes (66,67%), Klebsiella sp. (100%), and Shigella sp. (100%).

Conclusion: levofloxacin is sensitive against bacteria that cause UTIs in type 2 DM patients with a percentage of 95,45%.

Keywords: Diabetes mellitus; Urinary Tract Infections; Levofloxacin.

Abstrak

Latar Belakang: *Diabetes melitus (DM) merupakan penyakit gangguan metabolik menahun yang memiliki karakteristik berupa hiperglikemia, kondisi ini dapat berkaitan dengan kerusakan organ jangka panjang, disfungsi organ, dan kegagalan berbagai organ dalam tubuh. DM tipe 2 mengakibatkan tingginya kadar glukosa dalam urin dan penekanan sistem imun, sehingga dapat menjadi faktor predisposisi terhadap infeksi saluran kemih (ISK). Terapi antibiotik yang dapat diberikan pada ISK adalah levofloksasin yang efektif terhadap banyak jenis bakteri penyebab ISK. Penelitian ini bertujuan untuk mengetahui pola sensitivitas antibiotic levofloksasin terhadap bakteri penyebab infeksi saluran kemih pada pasien diabetes melitus tipe 2.*

Metode*: Penelitian menggunakan metode deskriptif dengan total sampel sebanyak 22 isolat bakteri yang telah diisolasi dari pasien ISK dengan DM tipe 2. Isolat bakteri merupakan koleksi Laboratorium Mikrobiologi Fakultas Kedokteran Universitas Tanjungpura. Penelitian dilaksanakan pada bulan Juni – Oktober 2019. Pengujian sensitivitas antibiotik menggunakan metode difusi cakram dan menggunakan tabel Clinical & Laboratory Standards Institute (CLSI) sebagai panduan kriteria.*

Hasil: *levofloksasin sensitif terhadap bakteri ISK sebesar 95,45% dengan jumlah isolat sebanyak 21 dari 22 isolat uji. Pola sensitivitas levofloksasin yang bersifat sensitif pada jenis bakteri yaitu Escherichia coli (100%), Pseudomonas aeruginosa (100%), Enterobacter aerogenes (66,67%), Klebsiella sp. (100%), dan Shigella sp. (100%).*

Kesimpulan: *levofloksasin sensitif terhadap bakteri penyebab ISK pada pasien DM tipe 2 dengan persentase sebesar 95,45%.*

Kata kunci: *Diabetes Melitus; Infeksi Saluran Kemih; Levofloksasin.*

IINTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder caused by the pancreas that unable to produce insulin adequately or the body unable to use the insulin effectively. As a result, there is an increase in the concentration of glucose in the blood (hyperglycemia).[1] Uncontrolled condition of hyperglycemia will be a trigger factor of DM vascular complications, such as diabetic retinopathy, diabetic nephropathy, heart disease, and diabetic neuropathy.[2] The prevalence of DM worldwide has increased, with type 2 DM being the most common, approximately as much as 85% of DM patients.[3] Type 2 DM in Indonesia recorded an increase in 2013 which became 6.9%, whereas previously in 2007 it was only with a percentage of 5.7%.[1] The incidence rate of type 2 DM in West Kalimantan itself was recorded as having a percentage of 0.8% with an estimated number of 24,581 cases.[4]

Chronic hyperglycemia in DM is associated with long-term damage, dysfunction, and failure of various organs in the patient's body, such as eyes, genitourinary system, nerves, heart, and blood vessels. Hyperglycemia also suppresses the immune system by making conditions tolerant to infection due to neutrophil dysfunction. High blood glucose levels can cause the kidneys to be unable to filter and reabsorb glucose and glucose is released with the urine, this condition is called glucosuria. High urine glucose levels and suppression of the immune system are predisposing factors for infections, especially urinary tract infections (UTI).[5] Clinical epidemiological studies have reported that UTIs were found to be more common in DM patients compared to those without DM.[6] The risk of UTI occurrence in DM patients worldwide is estimated at 60% and 47% in Indonesia.[7]

UTI is an infection that often affects men and women of all ages. In general, UTI is caused by Uropathogenic Escherichia coli (UPEC), which is the most common bacteria found in symptomatic and asymptomatic UTI patients. Other bacteria that are often found are Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus.[8] The incidence of UTI globally reached 35-45%, while the incidence of UTI in Indonesia was around 40-60%.

According to The Infectious Disease Society of America (IDSA), antibiotic therapy for UTIs with unknown causative bacteria are cephalosporin, fluoroquinolones, and aminoglycoside.[9] Currently, fluoroquinolones are still recommended as a therapy and prophylactic antibiotic for UTIs because fluoroquinolones have strong antibacterial activity against the bacteria that cause UTIs. Ciprofloxacin is the most widely used fluoroquinolone. However, recently there have been many reports about the resistance of the fluoroquinolone group as a prophylaxis or therapy for UTIs, especially ciprofloxacin, which ranged from 20%-30%.[10] Another fluoroquinolone antibiotic is levofloxacin, which is including of third generation quinolone antibiotic. Levofloxacin is effective in UTIs caused by many types of bacteria. Levofloxacin has excellent activity against Gram negative bacteria and moderate to good activity against Gram positive bacteria.[11] The broad use of antibiotics for the treatment of infectious diseases such as bacteria that cause UTIs in DM patients can lead to bacterial resistance. In 2015, the research at Dr. Soetomo Hospital (Surabaya) showed that levofloxacin was sensitive to bacteria that cause UTIs by 31.6%, while 59% at Dr. Cipto Mangunkusumo Hospital (Jakarta).[12] Research at the Ulin Hospital (Banjarmasin) had reported that 84.6% sensitive and 3.8% resistant to this antibiotic.[13] At the Raden Mattaher Hospital (Jambi) in 2016 was also reported that 60.87% sensitive and the 32.61% resistant to levofloxacin.[14] The difference in sensitivity values is due to the presence of bacterial resistance in each of these areas.

Antibiotic sensitivity data plays an important role in making decisions on the management of a disease. In West Kalimantan, especially in Pontianak, there is no data regarding the pattern of levofloxacin sensitivity in DM patients with UTIs. Therefore, this study aimed to provide an overview of levofloxacin sensitivity in UTIs patients with type 2 DM and this is the first data regarding levofloxacin sensitivity in Pontianak. This data is expected to be useful as a basis for therapy for clinicians in hospitals.

METHODS

Bacterial isolates were collections of the microbiology laboratory at the Faculty of Medicine, Tanjungpura University, which were obtained from type 2 DM patients with urinary tract infections (UTIs) in Sultan Syarif Mohamad Alkadrie Hospital, Pontianak.[15] The research was conducted from June to October 2019 in Microbiology Laboratory, Faculty of Medicine, Universitas Tanjungpura. The bacterial culture stocks were in glycerol and tryptic soy broth medium (60:40) and stored in the freezer -35^{o}C. The bacteria were re-cultured using the streak plate method on Eosin Methylene Blue Agar (EMBA) media prior to testing. Bacterial confirmation based on appearance on colony morphology, cells and biochemical properties test. The research was approved by the Health Research Ethics Committee, Faculty of Medicine of Universitas Tanjungpura with no. 3961/UN22.9/PG/2019.

The re-cultured bacteria aged 18-24 hours were then carried out by making a bacterial suspension in 0.9% sterile NaCl solution, followed by measuring the absorbance at a wavelength of 625 nm compared to the absorbance value of 0.5 Mc Farland solution which has an absorbance value ranged from 0.08 to 0,13.

Levofloxacin sensitivity test used the disc diffusion method by inoculating the bacteria on MHA media using a sterile swab. The bacterial suspension is taken by dipping a sterile swab into the bacterial suspension tube and then smearing it on the surface of the MHA using the smear method. Levofloxacin antibiotic disks were placed on the surface of the agar at a dose of 5µg/disk. Incubation was carried out at 37°C for 24 hours. The inhibition zone formed was observed and measured with calipers. Interpretation of test results based on the Clinical and Laboratory Standards Institute (CLSI) for the antibiotic of levofloxacin. Interpretation results were categorized as sensitive if they had an inhibition zone of ≥17 mm, intermediate (14-16 mm), and resistant (≤13 mm).

RESULTS

A total of 22 clinical bacterial isolates from T2DM Patients were success to reculture for sensitivity pattern analysis which consist of 12 isolates of Escherichia coli, 5 isolates of Pseudomonas aeruginosa, 3 isolates of Enterobacter aerogenes, 1 isolates of Klebsiella sp., and 1 solates of Shigella sp.. The characteristic of clinical bacterial isolates from T2DM Patients can be seen in Table 1.

Table 1. The Characteristic of Clinical Bacterial Isolates from T2DM Patients

No.	Code	Colonies color	Media's color	Shape of Cell	Bacteria
1	6	Deep purple	Purple	Basil (-)	*Escherichia coli*
2	11	Whitish purple	Light purple	Basil (-)	*Pseudomonas aeruginosa*
3	38B	Pink with *shiny surface*	Purplish pink	Basil (-)	*Enterobacter aerogenes*
4	42A	Deep purple	Purple	Basil (-)	*Escherichia coli*
5	45	Deep purple	Purple	Basil (-)	*Escherichia coli*
6	58A	Deep purple	Purple	Basil (-)	*Escherichia coli*
7	63A	White	Brown	Basil (-)	*Shigella sp.*
8	63B	Deep purple	Purple	Basil (-)	*Escherichia coli*
9	67B	Deep purple	Purple	Basil (-)	*Escherichia coli*
10	72A	Whitish purple	Light purple	Basil (-)	*Pseudomonas aeruginosa*
11	73A	Deep purple	Purple	Basil (-)	*Escherichia coli*
12	73B	Pink with *shiny surface*	Purplish pink	Basil (-)	*Enterobacter aerogenes*
13	74B	Whitish purple	Light purple	Basil (-)	*Pseudomonas aeruginosa*
14	82	Whitish purple	Light purple	Basil (-)	*Pseudomonas aeruginosa*
15	88	Purple	Purple	Basil (-)	*Klebsiella sp.*
16	89A EMB	Deep purple	Purple	Basil (-)	*Escherichia coli*
17	95 EMB	Deep purple	Purple	Basil (-)	*Escherichia coli*
18	96 EMB	Pink with *shiny surface*	Purplish pink	Basil (-)	*Enterobacter aerogenes*
19	99 EMB	Deep purple	Purple	Basil (-)	*Escherichia coli*
20	102A EMB	Deep purple	Purple	Basil (-)	*Escherichia coli*
21	103A	Whitish purple	Light purple	Basil (-)	*Pseudomonas aeruginosa*
22	103B	Deep purple	Purple	Basil (-)	*Escherichia coli*

A total of 21 from 22 bacterial isolates (95.45%) were sensitive to levofloxacin and 1 isolate was resistant to levofloxacin (4.55%). The levofloxacin sensitivity pattern to each each bacterial species were 100% sensitive to Escherichia coli, Pseudomonas aeruginosa, Klebsiella sp., and Shigella sp., but 66.67% sensitive and 33,33% resistant to Enterobacter aerogenes. The inhibition zone formed ranged from 0-32.40 mm. The results of the levofloxacin sensitivity test can be seen in Table 2.

Table 2. Levofloxacin Sensitivity Pattern

No.	Code	Bacteria	Inhibition Zone Diameter (mm)	Results
1	6	*Escherichia coli*	30,00	Sensitive
2	11	*Pseudomonas aeruginosa*	22,52	Sensitive
3	38B	*Enterobacter aerogenes*	0,00	Resistant
4	42A	*Escherichia coli*	25,60	Sensitive
5	45	*Escherichia coli*	27,29	Sensitive
6	58A	*Escherichia coli*	28,00	Sensitive
7	63A	*Shigella sp.*	30,92	Sensitive
8	63B	*Escherichia coli*	27,78	Sensitive
9	67B	*Escherichia coli*	16,08	Sensitive
10	72A	*Pseudomonas aeruginosa*	32,40	Sensitive
11	73A	*Escherichia coli*	30,22	Sensitive
12	73B	*Enterobacter aerogenes*	31,00	Sensitive
13	74B	*Pseudomonas aeruginosa*	24,46	Sensitive
14	82	*Pseudomonas aeruginosa*	25,56	Sensitive
15	88	*Klebsiella sp.*	20,32	Sensitive
16	89A EMB	*Escherichia coli*	26,77	Sensitive
17	95 EMB	*Escherichia coli*	27,30	Sensitive
18	96 EMB	*Enterobacter aerogenes*	25,61	Sensitive
19	99 EMB	*Escherichia coli*	27,70	Sensitive
20	102A EMB	*Escherichia coli*	28,70	Sensitive
21	103A	*Pseudomonas aeruginosa*	26,85	Sensitive
22	103B	*Escherichia coli*	22,34	Sensitive

DISCUSSIONS

Generally, UTI is caused by Escherichia coli which is the bacteria most often isolated from UTI patients and other bacteria that can also be found are Klebsiella, Proteus mirabilis, Pseudomonas, Enterococcus, and Staphylococcus saprophyticus[8]. The distribution of bacterial species in this study indicated that Escherichia coli was the dominant bacteria isolated from UTI patients. The results showed that there were 54.55% of Escherichia coli isolates, followed by 22.73% of Pseudomonas aeruginosa, 13.64% of Enterobacter aerogenes, 4.54% of Klebsiella sp., and 4.54% of Shigella sp. The sensitivity test results showed that levofloxacin was sensitive to 21 isolates (95.45%) and resistant to 1 isolate (4.55%). Another study using ciprofloxacin against the same isolates showed 63.63% sensitive, 4.54% intermediate and 9.09% resistant.[16] Previous research conducted at Ulin Hospital in Banjarmasin and at Raden Mattaher Hospital in Jambi also showed results with percentages close to 84.6% and 83.3%, respectively.[13] However, in contrast to research data reported by Dr. Soetomo Hospital, Surabaya, namely the sensitivity of levofloxacin to bacteria that cause UTIs is classified as low, i.e. 31.6%.[12]

The results of the presence of resistant bacteria in this study were probably caused by the intrinsic factors of these bacteria. Some intrinsic factors that are thought to influence are chromosomal resistance, extrachromosomal resistance, and cross-resistance. Chromosomal resistance is the resistance of bacteria to antibiotics that have resistance genes on the chromosome, for example due to spontaneous mutations in DNA loci that control susceptibility to certain drugs. In extrachromosomal resistance factors, bacteria contain extrachromosomal genetic elements called plasmids. Factor R is a group of plasmids that carry resistance genes to one or more antibiotics and

heavy metal compounds. Plasmid genes for antibiotic resistance control the formation of enzymes that can interfere with the action of antibiotics. The explanation for cross-resistance is that bacterial populations that are resistant to a particular drug may also be resistant to other drugs that may have a similar mechanism of action. This, for example, occurs in drugs whose chemical composition is almost the same, although sometimes there is also cross-resistance to two drugs with completely different chemical structures.[17] The self-defense system created by bacteria can be caused by continuous exposure to antibiotics.[18]

The results of this study showed that there is one bacterial isolate i.e. Enterobacter aerogenes which is resistant to levofloxacin, this resistant condition is thought to be caused by a bacterial defense mechanism. The previous study informed that Enterobacter aerogenes had an efflux or pump mechanism that can pump out antibiotic compounds including the fluoroquinolones out of cells so that antibiotics cannot work to stop bacterial growth. Enterobacter aerogenes had the efflux genes EefABC and AcrAB-TolC which can secrete about 80-90% of levofloxacin molecules during the first 10-15 minutes, this process is energy dependent and requires membrane energy (proton driving force).[19] Inappropriate use of antibiotics such as consumption that is not in accordance with the recommended time, low antibiotic doses, wrong choice of antibiotics, or wrong diagnosis can be a factor causing resistance.[20,21]

CONCLUSION

Levofloxacin antibiotic sensitivity test on 22 isolates of UTI-causing bacteria in type 2 DM patients showed that levofloxacin was sensitive as a therapy for UTI in type 2 DM patients (95,45%).

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