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Antibiotic Sensitivity Pattern of Bacterial Isolates Among Diabetic Outpatients with Urinary Tract infection in Pontianak

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Diabetic patients are associated with a higher risk of infection. The research purposed to identify antibiotic susceptibility patterns among diabetic outpatients with urinary tract infection in Pontianak. An experimental study was performed for 13 bacterial isolates of diabetic outpatients with urinary tract infection in the Clinic of Diabetes Mellitus, Sultan Syarif Mohamad Alkadrie Hospital, Pontianak. The disc diffusion method was used to perform the susceptibility of antibiotics to the bacterial isolates. Among 13 isolates, the most common causative agent of urinary tract infection was *Escherichia coli* (53.85%), followed by *Pseudomonas aeruginosa* (30.77%), *Klebsiella* spp., and *Enterobacter aerogenes* were 7.69%. Most isolates of bacteria of the study had a high sensitivity to Cefepime (92.31%), then followed by Levofloxacin, Amikacin, and Meropenem for 84.62%. The study revealed low sensitivity of bacteria to Amoxicillin/Clavulanate, Co-Trimoxazole, Cephazoline, and Ceftriaxone (30.77%, 23.08 %, 23.08%, 23.08%, respectively). All bacterial isolates had high resistance to Ampicillin. Moreover, multidrug resistance observed among bacterial isolates.

Key words: antibiotic susceptibility, diabetes, urinarytract infections

Pasien dengan diabetes memiliki risiko tingg mengalami infeksi. Penelitian ini bertujuan untuk mengetahui pola sensitivitas antibiotik pada pasien rawat jalan diabetes mellitus dengan infeksi saluran kemih di Pontianak. Penelitian ini dilakukan pada 13 isolat bakteri dari pasien diabetes dengan infeksi saluran kemih di Klinik Diabetes Mellitus, Rumah Sakit Sultan Syarif Mohamad Alkadrie, Pontianak. Uji sensitivitas antibiotik dilakukan menggunakan metode difusi cakram. Dari total 13 isolat bakteri, penyebab terbanyak dari infeksi saluran kemiha dalah *Escherichia coli* (53,85%), kemudian *Pseudomonas aeruginosa* (30,77%), *Klebsiella* spp., dan *Enterobacter aerogenes* sebanyak 7,69%. Hampir seluruh isolate bakteri menunjukkan sifat sensitif terhadap Cefepime (92,31%), kemudian Levofloxacin, Amikacin, dan Meropenem sebesar 84,62%. Sensitivitas rendah terlihat pada Amoxicillin/Clavulanate, Co-Trimoxazole, Cephazoline, dan Ceftriaxone (30,77%, 23,08 %, 23,08%, 23,08%, secara berurutan). Semua isolate bakteri menunjukkan resistensi terhadap Ampicillin dan resistensi terhadap lebih dari satu jenis antibiotik.

Kata kunci: diabetes, infeksi saluran kemih, sensitivitas antibiotik

Diabetesis a severe chronic metabolic disorder characterized by high blood glucose because of insulin production disorder, the body's inability to insulin utilization, or both. The prevalence of diabetes has increased during the last few decades (World Health Organization 2016; Gutema *et al.* 2018). Over time diabetesmay developorgan failure and suppressed the immune system that leads to an increase in the risk of infection. Urinary tract infection remains to be the most common infection diagnosed in diabetic patients. Urinary tract infection in diabetes is 46.9 per 1,000 people/year, which is higher than non-diabetes (29.9 per 1,000 people/year) (Fowler 2008; Hirji et al. 2012). Stage of urinary tract infection ranging from asymptomatic, dysuria to pyelonephritis. Diabetic patients are 15 times have a higher risk for hospitalization due to pyelonephritis (Saleem and Daniel 2011). Therefore, it is a significant problem for patients with diabetes to get appropriate treatment.

The successful therapy for urinary tract infection depends on the identification of microbial agents and the selection of antimicrobial against them (Gutema *et al.* 2018).The mainbacteria associated with urinary tract infection in diabetes are *Escherichia coli, Enterococcus* spp*., Klebsiella* spp*., Proteus* spp*., Pseudomonas aeruginosa, Staphylococcus aureus,* and coagulase-negative streptococcus. Other studies showed that fungi, namely, *Candida* spp. and *Actinomyces* spp*.,* also have a role as urinary tract infection agents in diabetes mellitus(Borj *et al.* 2017; Gutema *et al.* 2018).

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Antibiotic susceptibility is diverse among species and areas. Therefore, determining the sensitivity of antibiotics to bacterial isolates is essential. Furthermore, there is an increase in the antibiotic resistance prevalence due to the widespread and indiscriminate use of broad-spectrum antibiotics (Al-tulaibawi 2019). Data showed about 30% of urinary tract infection bacterial agents are resistant to ciprofloxacin and levofloxacin (Triono and Purwoko 2012; Rahman 2017). Thus, this study was performed to provide local data about the susceptibility pattern of antibiotics among diabetic outpatients with urinary tract infection in Pontianak.

MATERIALS AND METHODS

Research Design. An experimental study was conducted at the Microscopic Laboratory, Faculty of Medicine, Universitas Tanjungpura, Pontianak, during the period November 2019 to July 2020. The research procedures were approved by Ethics Committee of the Faculty of Medicine, Universitas Tanjungpura.

Bacterial Isolates. Bacterial isolates were derived from diabetic outpatients with urinary tract infection in the Clinic of Diabetes Mellitus, Sultan Syarif Mohamad Alkadrie Hospital, Pontianak. All patients were from Pontianak. Isolation of bacteria were inoculated in MacConkey Agar (Merck) and identification of bacteria by biochemistry test. All procedures were done by previous study. A total of 13 bacterial isolates were inoculated in MacConkey (Merck) agar plates using a standard inoculating loop for bacterial regrowth and incubated (Memmert) at 37 °C for 24 hours.

Antibiotic Susceptibility Test. McFarland 0.5 of bacterial suspensions were inoculated in Mueller Hinton Agar (Merck) to undergo antibiotic susceptibility testing using the disk diffusion method. Sterile cotton swab was dipped in the suspension and the excess liquid pressed. The sterile cotton swab was swab on the agar plate surface and repeated three times by rotated 60 °C of the plate. Antibiotic disks were placed on inoculated agar surface and incubated at 37 °C for 24 hours. The inhibition zone was examined according to the Clinical and Laboratory Standard Institute guidelines (Clinical and Laboratory Standards Institute 2020). Antibiotic agents that were used are Co-trimoxazole (SXT, 25µg, Oxoid), Ciprofloxacin (CIP, 5 µg, Oxoid), Levofloxacin (LEV, 5 µg, Oxoid), Nitrofurantoin (F, 300 µg, Oxoid), Amikacin (AK, 30 µg, Oxoid), Ampicillin (AMP, 10 µg, Oxoid), Amoxicillin/Clavulanate (AMC, 30 µg, Oxoid), Cephazoline (KZ., 30 µg, Oxoid), Ceftriaxone (CRO, 30 µg, BD BBL), Cefepime (FEP, 30 µg, BD BBL), Gentamicin (GM., 10 µg, BD BBL), Meropenem (MEM, 10 µg, BD BBL), and Tobramycin (NN., 10 µg, BD BBL).

RESULTS

Generally, antibiotic susceptibility patterns for urinary bacterial isolates from diabetic patients showed high sensitivity to Cefepime (92.31%), then followed by Levofloxacin, Amikacin, and Meropenem for 84.62% each. The study revealed low sensitivity of bacteria to Amoxicillin/Clavulanate, Co-Trimoxazole, Cephazolineand Ceftriaxone (30.77%, 23.08 %, 23.08%, 23.08%, respectively). All bacterial isolates had high resistance to Ampicillin. *E. coli,* as the main causative agent in the study, was sensitive to Amikacin and Cefepime (100%, for each). *P. aeruginosa* was sensitive to Levofloxacin, Amikacin, and Meropenem (100%, for each), followed by Ciprofloxacin, Cefepime, Gentamicin, and Tobramycin (75%, for each). *E. pyogenes* were100% sensitive to Co-Trimoxazole, Levofloxacin, Amoxicillin/Clavulanate, and Cefepime. At the same time, *Klebsiella* spp. appeared sensitive to Cefepime and Meropenem (100%, for each). Detail of antibiotic sensitivity profile and inhibition zone diameter, as seen in Table 1 and Table 2, respectively.

DISCUSSION

Globally, antibiotic resistance rates are on the increase. Meanwhile, antibiotic sensitivity is a primary concern in the treatment of patients with infection. Patients with diabetes are prone to have an infection, commonly urinary tract infection because of the impaired immune response, dysfunctional bladder, and other mechanisms (Alrwithey *et al.* 2017). Other studies demonstrated that diabetic patients with urinary tract infections are vulnerable to have resistant pathogens as the causative agent (Nitzan *et al.* 2015). Our study revealed bacteria that cause urinary tract infection in patients with diabetes mellitus, namely *E. coli*(7/13), *Klebsiella* spp. (1/13), *E. aerogenes* (1/13), and *P. aeruginosa* (4/13). Several studies reported that *E. coli*is the most common bacteria in urinary tract infection in diabetic or non-diabetic patients (Nitzan *et al.* 2015; Borj *et al.* 2017; Gutema *et al.* 2018; Al-tulaibawi 2019).

Table 1 Antibiotic sensitivity profile of bacterial isolates from diabetic patients with urinary tract infection

Antibiotic sensitivity n (%)														
Bacteria	***n**	**SXT**	**CIP**	**LEV**	**F**	**AK**	**AMP**	**AMC**	**FEP**	**KZ**	**CRO**	**GM**	**MEM**	**NN**
Escherichia coli	7	2 (28.57)	5 (71.43)	6 (85.71)	1 (14.29)	7 (100)	0 (0)	3 (42.86)	7 (100)	3 (42.86)	1 (14.29)	5 (71.43)	6 (85.71)	6 (85.71)
Pseudomonas aeruginosa	4	0 (0)	3 (75)	4 (100)	0 (0)	4 (100)	0 (0)	0 (0)	3 (75)	0 (0)	2 (50)	3 (75)	4 (100)	3 (75)
Klebsiella spp.	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
Enterobacter pyogenes	1	1 (100)	(0)	1 (100)	(0)	(0)	(0)	1 (100)	1 (100)	(0)	(0)	(0)	(0)	(0)
Total	13	3 (23.08)	8 (61.54)	11 (84.62)	1 (7.69)	11 (84.62)	0 (0)	4 (30.77)	12 (92.31)	3 (23.08)	5 (38.46)	8 (61.54)	11 (84.62	9 (69.23)

*n: Number of isolates

SXT: Co-trimoxazole, CIP: Ciprofloxacin, LEV: Levofloxacin, F: Nitrofurantoin, AK: Amikacin, AMP: Ampicillin, AMC: Amoxicillin/Clavulanate, KZ.: Cephazoline, CRO: Ceftriaxone, FEP: Cefepime, GM.: Gentamicin, MEM: Meropenem, NN.: Tobramycin.

Table 2 Inhibiton zone diameter of disk diffusion among bacterial isolates from diabetic patients with urinary tract infection

Isolate	Inhibition Zone Diameter (mm)												
	SXT	CIP	LEV	F	AK	AMP	AMC	FEP	KZ	CRO	GM	MEM	NN
1	0	21.60**	22.52*	0	26.13*	7.02	9.33	28.67*	0	15.33	17.67*	25.33*	20.67*
2	23.60*	26.08*	25.60*	10.40	23.08*	0	8.18	30.27*	9.50	22.12**	11.06	29.53*	15.13*
3	18.82*	27.15*	27.29*	17.23*	20.02*	0	26.84*	33.66*	26.40*	30.60**	16.68*	30.33*	16.68*
4	0	11.28	16.08	14.66	28.67*	0	0	25.67*	0	19.33	19.17*	19.03	20.41*
5	21.02	32.18*	32.40*	0	19.16*	0	11.17	32.00*	0	30.33*	17.50*	29.00*	16.67*
6	0	24.32*	24.46*	0	17.33*	0	9.16	13.83	0	6.67	0	30.66*	9.83
7	0	14.94	20.32**	14.73	10.80	0	11.56	30.00*	8.42	28.02**	0	28.00*	10.80
8	0	27.44*	26.77*	16.17**	19.55*	11.66	10.02	34.32*	23.66*	34.84*	15.16*	29.50*	15.68*
9	0	23.31*	27.30*	15.14**	17.58*	8.52	20.32*	31.50*	0	24.00**	14.00**	30.00*	14.00**
10	21.02*	23.16	25.61*	0	16.87**	9.32	20.96*	30.20*	0	20.21	14.32**	18.04	14.06**
11	0	28.29*	27.70*	16.06**	18.03*	12.51	27.53*	31.62*	28.52*	28.59**	16.11*	29.08*	15.27*
12	0	27.68*	26.85*	0	19.57*	12.06	26.11	32.80*	0	27.38*	16.51*	30.72*	17.38*
13	0	16.16	22.34*	16.06**	19.06*	0	9.22	26.24*	9.04	21.62**	17.02*	28.32*	17.32*

*Sensitive, **Intermediate, Resistant
Isolate no.1,5,6,12 *P. aeruginosa;* no.2-4,8,9,11,3 *E. coli;* no.7 *Klebsielasp;* no.10 *E. aerognenes*
SXT: Co-trimoxazole, CIP: Ciprofloxacin, LEV: Levofloxacin, F: Nitrofurantoin, AK: Amikacin, AMP: Ampicillin, AMC: Amoxicillin/Clavulanate, KZ.: Cephazoline, CRO: Ceftriaxone, FEP: Cefepime, GM.: Gentamicin, MEM: Meropenem, NN.: Tobramycin.

Table 1 demonstrated that all bacteria isolates are resistant to Ampicillin (100%). Ampicillin is an antibiotic effective for Gram-positive and Gram-negative microorganisms. However, some microorganisms develop resistance to Ampicillin. Studies have shown an increasing trend in ampicillin-resistance (Aamodt *et al.* 2015; Richey *et al.* 2015). Contradict to other studies, Nitrofurantoin showed the second rank of the highest antibiotic resistance (92.31%)(Gardiner *et al.* 2019; Zubair and Shah 2019).

Table 2 showed majority isolates have resistant to 5 antibiotics (30.76%) and 2 isolates demonstrated resistant to 9 antibiotics (15.38%). There is an increasing trend in antimicrobial resistance among uropathogenic. The primaryantibiotic resistance mechanism for Gram-negatives are the production of β-lactamases and frequently aminoglycoside modifying enzymes (Khoshnood *et al.* 2017; Bitsori and Galanakis 2019). Bacteria classified as *Enterobacteriaceae* with sensitivity test results resistance or intermediate towards third-generation cephalosporin antibiotic should be tested for the production of Extended Spectrum ß-lactamases (ESBL). A previous study in Dr.Soetomo Hospital Surabaya found a more significant rate of ESBL producing *E.coli* compare to non-ESBL producing *E. coli* (Fitri *et al.* 2015). Pathogensthat produce ESBL represent resistance to third-generation cephalosporin, monobactam, as well as to newer ß-lactam antibiotics (Bitsori and Galanakis 2019). Further test is needed to reveal ESBL bacteria in this study.

Based on our study, Cefepime was reported as an antibiotic for urinary tract infection with the highest sensitivity compared to others (92.31%). Cefepime is classified as beta-lactam, fourth-generation cephalosporin antibiotic. It is used to treat uncomplicated pyelonephritis as second-line therapy and as an alternative therapy in urosepsis, renal diseases, and Extended-Spectrum Beta-Lactamases bacteria (Baldwin *et al.* 2008; Seputra *et al.* 2015; Bonkat *et al.* 2018; Kim *et al.* 2018).

E. coli isolates exhibited sensitivity towards Amikacin and Cefepime (100% for each), followed by Levofloxacin, Meropenem, and Tobramycin (85.71%, for each), Ciprofloxacin (73.41%). Less sensitivity is shown towards Amoxicillin/Clavulanate, Cephazoline (42.86%, for each), Co-Trimoxazole (28.57%), Nitrofurantoinm and Ceftriaxone (14.29%, for each), whereas another study has shown the opposite result (Gutema *et al.* 2018; Al-tulaibawi 2019; Zubair and Shah 2019).

P. aeruginosa was shown as the highest sensitivity towards Levofloxacin, Amikacin, and Meropenem (100%, for each), Ciprofloxacin, Cefepime, Gentamycin, and Tobramycin (75%, for each) and less sensitivity for Ceftriaxone (50%). Other antibiotics do not affect *P. aeruginosa. P. aeruginosa* is known to have resistance towards multiple antibiotics, such as aminoglycoside, quinolones, and β-lactams through some mechanisms (Pachori *et al.* 2019; Pang *et al.* 2019). *P. aeruginosa* demonstrated intrinsically, acquired, and adaptive resistance(Pang *et al.* 2019).

Further research using a larger population or samples and different hospitals should be conducted. This study results may be used as data to improve the treatment of diabetic patients with urinary tract infections based on the pattern of antibiotic susceptibility. Antibiotic susceptibility pattern is required for the rational use of antibiotics and the prevention of resistant urinary pathogens. Furthermore, the rise of antibiotic resistance should be a significant concern for clinicians in treating diabetic patients with urinary tract infection, as demonstrated in this study.

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