Research Article

Nitrofurantoin Susceptibility Profile on Bacterial Isolates from Urinary Tract Infection in Patients with Diabetes Mellitus

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ABSTRACT

Background: Patients with diabetes mellitus have a higher risk of urinary tract infection (UTI) incidence than those without. One of the first-line therapy for UTI is nitrofurantoin. The emergence of drug-resistant UTIs is increasing both in community and healthcare setups. **Purposes:** Determine the nitrofurantoin susceptibility profile to bacteria causing UTI in diabetic patients. **Method:** A cross-sectional study was conducted at the Microscopic Laboratory, Faculty of Medicine, Universitas Tanjungpura, Pontianak, from February to November 2019. The antibiotic susceptibility testing was performed by the Kirby-Bauer disk diffusion method on 22 bacterial isolates. The samples were collected on a sterile urine pot, cultured on agar, and identified by biochemistry test, and bacterial isolates were stored at 2-8°C. The bacterial isolates were sub-cultured 24h before the susceptibility test. The susceptibility testing used a 300 µg nitrofurantoin disk. The diameter of zone inhibition was measured and classified based on Clinical and Laboratory Standard Institute guidelines. **Result:** The results showed that *Escherichia coli* was 41.67% susceptible (5/12*), Shigella sp*. was 100% intermediate (1/1), and *Enterobacter aerogenes* (3/3), *Pseudomonas aeruginosa* (1/1), *Klebsiella sp* (1/1) were 100% resistant. **Conclusion:** The majority of the causative bacteria for UTI in diabetic patients are resistant to nitrofurantoin, suggesting the use of nitrofurantoin should be reconsidered as an empirical antibiotic in Pontianak. Further study using a larger population should be conducted to describe a more extensive antibiotic susceptibility profile of diabetic patients with UTI in Pontianak.

Keywords: antibiotic susceptibility, diabetes mellitus, nitrofurantoin, urinary tract infection

INTRODUCTION

Urinary tract infection (UTI) is an infection of the urinary tract characterized by microorganism growth of more than 105 CFU/ml in urine. Most of UTI's etiology is *Escherichia coli, Proteus sp., Klebsiella sp., Staphylococcus sp., Enterococcus sp*., and *Pseudomonas sp* (1,2). Globally, the prevalence of UTI is 150 million every year (3). The prevalence of UTI in Indonesia is relatively high, predicted to be 90-100 out of 100,000 cases yearly or about 180,000 new cases annually (4).

The prevalence of UTI is affected by age, gender, bacteriuria, and predisposing factors that cause structural changes to the urinary tract, such as diabetes mellitus (5). Diabetes mellitus is a metabolic disease characterized by hyperglycemia caused by insulin secretion disorder,

insulin function disorder, or both (6). Diabetes mellitus patients are known to have a higher incidence of UTI (1).

The increased UTI risk in diabetic patients might be caused by bladder nerve damage, affecting the ability to sense the presence of urine, thus allowing urine to stay longer in the bladder and increasing the probability of infection. Additionally, the high glucose levels in the urine play a role in the growth of the bacteria, thus increasing the risk of infection. Chronic diabetes mellitus may also result in abnormalities in the immune system that increase the risk of developing various infections (7). In Indonesia, the urinary tract infection incidence in diabetic patients is 47% (8). Meanwhile, 83% of diabetic patients are reported to have UTI in Pontianak (9).

Urinary tract infection management is through antibiotic treatment, and one of the first-line antibiotics is nitrofurantoin (10). Nitrofurantoin is a nitrofuran antibiotic with a bactericidal mechanism for Escherichia coli and other Gram-negative bacteria. Nitrofurantoin may be used as urinary tract infection therapy in adults as prophylaxis in children, but it is not recommended for patients with kidney function disorders (11).

Drug-resistant UTIs are emerging in community and healthcare setups (12). Some studies reported the effects of drug resistance are deaths, length of hospitalization, and healthcare costs (13). Therefore, it is essential to establish an antibiotic susceptibility profile. The study in RSUD dr.Soetomo showed that most bacteria found are *Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, and the susceptibility to nitrofurantoin is 47%. Meanwhile, the nitrofurantoin susceptibility to *Escherichia coli* in RSUP Sanglah in Bali is 90%. The susceptibility test in RSUP H. Adam Malik in Medan showed a susceptibility percentage of *Escherichia coli* is >60% (14). Other studies showed nitrofurantoin susceptibility to Escherichia coli in patients with diabetes mellitus is 67% (15). From those studies, it is known that UTI-causative bacteria have various susceptibility profiles to nitrofurantoin. To our knowledge, there was no data about nitrofurantoin susceptibility profile in Pontianak, especially for diabetes mellitus patients with UTI infection. Therefore, this study was conducted to determine the nitrofurantoin susceptibility profile of UTI among diabetic patients.

METHODS

The study was a descriptive cross-sectional study conducted at the Microscopic Laboratory, Faculty of Medicine, Universitas Tanjungpura, Pontianak, from February 2019 to November 2019. The urine samples from diabetes mellitus inpatients and outpatients with UTI were collected on a sterile urine pot, cultured on Mac Conkey agar, identification of species by biochemistry test, and the bacterial isolates were stored at 2-8ºC in the Microscopic Laboratory, Faculty of Medicine, Universitas Tanjungpura. All procedures were performed in the previous study (9). The bacterial isolates were sub-cultured 24h before the susceptibility test. Bacterial isolates were streaked on Eosin Methylene Blue agar (Merck, USA) using a standard inoculating loop, and incubated at 37°C for 24 hours. Then, the colony was characterized by colony form and Gram staining. A total of 22 bacterial isolates were included in the study.

McFarland 0.5 standard solution was made by mixing 9.95 ml of 1% H_2SO_4 and 0.05 ml of 1% $BaCl_2$. The solution was homogenized using a vortex, and the turbidity equals 1.5×10^8 CFU/ml (16). The solution was then measured for Optical Density (OD) using

spectrophotometry at 625 nm, and the absorbance value was 0.08-0.13 (17). One to three single colonies suspended into 5 ml of 0.9% NaCl as bacterial suspension. The turbidity of bacterial suspension was compared with the absorbance value of McFarland 0.5 standard through spectrophotometry.

The antibiotic susceptibility test used the Kirby-Bauer disk diffusion method. The 0.5 McFarland of bacterial suspensions were inoculated to Mueller-Hinton Agar (Merck, USA) with a streak plate method using a sterile cotton swab. Nitrofurantoin (300 µg, Oxoid, UK) antibiotic disks were placed on an inoculated agar surface and incubated for 24 hours at 37°C (16–18). The clear zone of inhibition was measured using a caliper and interpreted according to Clinical and Laboratory Standard Institute guidelines as susceptible (≥17 mm), intermediate (15-16 mm), and resistant (≤14 mm) (16). The study was approved by The Ethical Committee of the Faculty of Medicine, Universitas Tanjungpura (No.3961/UN22.9/DL/2019).

RESULTS

The susceptibility testing for nitrofurantoin was conducted on 22 bacteria isolates. The bacteria isolates used in this study are *Escherichia coli* (12 isolates, 54.55%) and followed by *Pseudomonas aeruginosa* (five isolates, 22.73%). *Enterobacter aerogenes* were found in three isolates (22.73%). *Shigella sp*. and *Klebsiella sp*. were found in one isolate each (4.55%) (9). The susceptibility testing to nitrofurantoin showed that five of 12 *Escherichia coli* isolates were sensitive (41.67%). A total of three *Enterobacter aerogenes* isolates, five *Pseudomonas aeruginosa* isolates and one *Klebsiella sp*. isolate were found to be resistant to nitrofurantoin (100%). Meanwhile, one isolate of *Shigella sp*. results in intermediate to nitrofurantoin. The result of the susceptibility testing could be seen in Table 2.

Table 1. Nitrofurantoin susceptibility profile of bacterial isolates

Bacteria	N*	Susceptibility N (%)		
		Sensitive	Intermediate	Resistant
Escherichia coli	12	5 (41.67)	5 (41.67)	2 (16.67)
Enterobacter aerogenes	3	0	0	3 (100)
Shigella sp.	1	0	1 (100)	0
Klebsiella sp.	1	0	0	1 (100)
Pseudomonas aeruginosa	5	0	0	5 (100)
Total	22			

*: number of isolates

DISCUSSION

The study showed UTI in diabetic patients, the majority caused by *Escherichia coli* (54.55%), followed by *Pseudomonas aeruginosa* (22.73%) and *Enterobacter aerogenes* (13.64%), respectively. Most of the UTI-causative bacteria are enteric coliforms that inhabit the peri-urethral. The bacteria then ascend through the urethra into the bladder and cause UTI (19). Some studies also describe the etiology of UTI. Studies at Banjarmasin represent *Escherichia coli* (65.3%) and *Pseudomonas aeruginosa* (11.5%) as the majority etiology (20). This result is similar to another study in Kalyani, India, that shows the most common bacteria in diabetic patients with UTI is *Escherichia coli* (38.77%) (12).

Nitrofurantoin susceptibility testing towards *Escherichia coli* is that five isolates are sensitive, five are intermediate, and two are resistant. Another study in India demonstrated similar results. They revealed that about 56% of *Escherichia coli* isolates show a sensitive result to nitrofurantoin (21). The study's susceptibility test of *Enterobacter aerogenes* shows nitrofurantoin resistance towards all isolates (3/3 isolates). An antibiotic susceptibility study in Surabaya about urinary tract infection in diabetic patients shows the susceptibility profile of nitrofurantoin to *Enterobacter sp.* is 33% sensitive (15). The susceptibility test of *Shigella sp.* towards nitrofurantoin is 100% intermediate (1/1 isolate). This result differs from a study of *Shigella sp*. from feces samples, revealing that the isolates are susceptible to nitrofurantoin (93.1%) (22). The susceptibility testing to *Klebsiella sp.* results in resistance (1/1 isolate). A study in Ethiopia mentions that the resistance profile of *Klebsiella pneumonia* to nitrofurantoin is 55.6% (23). However, this study only performed a nitrofurantoin susceptibility test on one isolate of *Shigella sp.* and *Klebsiella sp*. Hence, the result may not truly represent the susceptibility profile. The differences in susceptibility patterns with other places are a common thing. Each of the bacteria species has its characteristic. Thus, antibiotic susceptibility profiles should be monitored to provide the prevalence of antibiotic-resistant bacteria and as clinician guidelines for empirical therapies at each city or hospital (24).

The susceptibility testing to *Pseudomonas aeruginosa* isolates shows resistance (5/5). These results were similar to a study in RSUD Arifin Achmad, Pekanbaru,that demonstrated that the susceptibility of *Pseudomonas aeruginosa* to nitrofurantoin is 0.8%. *Pseudomonas aeruginosa* is an intrinsically resistant bacteria to many antibiotics, including nitrofurantoin (25,26). The outer membrane permeability of *Pseudomonas aeruginosa* is hugely restricted (12-100-fold lower than that of *Escherichia coli*), which prevents antibiotics entrance (27). Nitrofurantoin resistance is caused by the inability of the antibiotic to penetrate the outer membrane and reach the targeted enzyme (28). A study testing two strains of *Pseudomonas aeruginosa* with nitrofurantoin failed to inhibit their growth due to its intrinsic resistance character. Instead, it enhances the biofilm formation in both strains of *Pseudomonas aeruginosa* (29). Another study of nitrofuran antibiotics (nitrofurazone, furazidine, nitrofurantoin, and nifuroxazide) within the range of sub-inhibitory concentrations also shows enhancement to the biofilm formation of *Pseudomonas aeruginosa*. Testing using nitric oxide generators (sodium nitroprusside and isosorbide mononitrate) gives a similar result. Thus, the increase in biofilm formation might be related to nitric oxide or other derivatives formed due to nitro and nitric oxide metabolism (30).

This study showed that most UTI-causative bacteria in diabetic patients resist nitrofurantoin. Only 41.67% of *Escherichia coli* isolates were shown to be susceptible, while the other bacteria isolates were resistant. Based on study results, the use of nitrofurantoin for UTI in diabetic patients should be reconsidered as an empirical antibiotic in Pontianak. The limitation of the study is the small number of samples and does not compare UTI in diabetic and non-diabetic patients. Further study using a larger population should be conducted to describe a more extensive antibiotic susceptibility profile of diabetic patients with UTI in Pontianak. Hence, defining the regional antibacterial susceptibility pattern for the clinician prescribing empirical antibiotics in daily practice is crucial.

CONCLUSION

The study of nitrofurantoin antibiotic susceptibility testing towards 22 bacterial isolates of diabetes mellitus patients with UTI demonstrated that three species are resistant to nitrofurantoin: *Enterobacter aerogenes, Klebsiella sp*., and *Pseudomonas aeruginosa*. Based on study results, the majority of the UTI-causative bacteria in diabetic patients are resistant to nitrofurantoin, suggesting the use of nitrofurantoin should be reconsidered as an empirical antibiotic in Pontianak.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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