

Comparison of Microbial Pattern Causing Urinary Tract Infection in Female Out- and Hospitalized Patients in Jakarta

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Urinary Tract Infection (UTI) is an infection in any part of the urinary system. Women are 3 times more likely to have UTI than men. The UTI accounts for 15% infection cases in outpatients and 24% cases in hospitalized patients. Although the most common cause of UTI is certain bacteria, but it was not easy to choose the appropriate antimicrobial therapy. Strategy for choosing empiric antimicrobial treatments for UTI in female out- and hospitalized patients should be based on the pattern of the causative organisms. The aim of this study was to understand the microbial pattern causing UTI in female out- and hospitalized patients in Jakarta. The UTI causative microorganisms were obtained from urine culture containing 100,000 cfu mL⁻¹. Twenty nine microorganisms were found as the causative agents of UTI in 317 pregnant women who came to six Community Health Centres (Puskesmas) in Jakarta: Makassar; Pulogadung, Cakung, Pasar Rebo, Duren Sawit and Kramat Jati for antenatal care. Twenty nine microorganisms were isolated from 114 urine samples of female hospitalized patients who were diagnosed of UTI. The samples were obtained from the Microbiology Laboratory Clinic of FKUI-RSCM. The most common microorganisms causing UTI in female out- and hospitalized patients were Gram negative bacteria. In female outpatients, *Klebsiella sp* was the most common causative bacteria (31%), followed by *Escherichia coli* (24.1%). In female hospitalized patients, *Escherichia coli* was the most common causative bacteria (30%), followed by *Candida sp* (24.1%) and *Klebsiella pneumoniae* (6.8%). There was more variation in the pattern of UTI causative organisms in hospitalized female patients in comparison to that of the outpatients. *Candida sp.* was only found in hospitalized UTI patients but not in outpatients.

Key words: causative agent, female, inpatient, outpatient, UTI

Infeksi Saluran Kemih (ISK) adalah infeksi yang terjadi pada berbagai bagian saluran kemih. Wanita memiliki risiko 3 kali lebih besar mengalami ISK dibandingkan pria. Kejadian ISK mencapai 15% pada pasien rawat jalan dan 24% pada pasien rawat inap di rumah sakit. Walaupun etiologi ISK yang paling umum adalah bakteri tertentu, tetapi tidak mudah untuk memilih antimikroba yang tepat untuk terapi. Strategi untuk memilih antimikroba empiris untuk ISK pada pasien wanita rawat jalan dan rawat inap harus didasarkan pada pola mikroorganisme dari etiologi. Tujuan penelitian ini adalah untuk melihat pola mikroorganisme penyebab ISK pada pasien wanita rawat jalan dan rawat inap di Jakarta. Pola mikroorganisme penyebab ISK didapatkan melalui metode kultur urin, dengan jumlah koloni 100.000 cfu mL⁻¹ urin. Sejumlah 29 bakteri didapatkan sebagai mikroorganisme penyebab ISK pada 317 wanita hamil yang melakukan pemeriksaan kehamilan di 6 puskesmas di Jakarta: Puskesmas Makassar, Pulogadung, Cakung, Pasar Rebo, Duren Sawit, dan Kramat Jati. Sebanyak 29 bakteri didapatkan dari 114 sampel urin wanita rawat inap yang didiagnosis sebagai ISK. Sampel urin diperiksa di Laboratorium Mikrobiologi Klinik FKUI-RSCM. Pola mikroorganisme penyebab terbanyak ISK pada rawat jalan dan rawat inap adalah mikroorganisme Gram negatif. Pada rawat jalan *Klebsiella pneumoniae* sebagai penyebab terbanyak ISK (31%), diikuti *Escherichia coli* (24,1%). Pada rawat inap *Escherichia coli* menjadi penyebab terbanyak ISK (30%), diikuti oleh *Candida sp* (24,1%) dan *Klebsiella pneumoniae* (6,8%). Pola mikroorganisme ISK pada rawat inap lebih bervariasi dibandingkan rawat jalan. *Candida sp* hanya ditemukan pada ISK rawat inap, tetapi tidak pada rawat jalan.

Kata kunci: ISK, penyebab infeksi, rawat jalan, rawat inap, wanita

Women are three times more likely to get urinary tract infection (UTI) than men, due to women's shorter urethra, sexual activity, pregnancy, and hormonal changes that occur very quickly (Mitchell *et*

al. 2002). Hormonal changes of both estrogen and progesterone during the menstrual cycle affect urodynamic. Estrogen deficiency can increase the resistance of the urethra as it can cause an increase in the threshold of the bladder or can boost the sensitivity of the receptors -adenoreseptor on the urethral muscle (Robinson *et al.* 2013). Estrogen deficiency also

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disturbed the roles of the hormone in stimulating the proliferation of *Lactobacillus* in the vaginal epithelium, reducing pH and avoiding vaginal colonization by Enterobacteriaceae, which are the main pathogens of the urinary tract (Raz R 2011). These conditions play an important role in the development of bacteriuria.

Around 35% of women aged between 20-50 years had experienced a UTI in their lifetime (Samirah *et al.* 2006). Most women aged about 24 years old (reproductive age) have experienced a UTI at least once in their life time. The high incidence of UTI during the reproductive age might be related to the high sexual activity. Therefore, the incidence of UTI in sexually active women are generally higher than non sexually active women.

Other risk factors for UTI include pregnancy, diabetes, obstruction of the urinary tract, older men with enlarged prostate, and other factors that disturb the physiology of the urinary tract (Mitchell *et al.* 2002). UTI acquired in hospitals are often associated with long term catheter use. Data from research conducted on healthcare-associated infections (HAIs) in Indonesia reported that the rate of hospital-acquired UTI was between 0.9 to 3.5% (Duerink *et al.* 2006).

Common bacteria causing urinary tract infection (UTI) include *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus sp*, *Enterobacter sp*, *Streptococcus sp*, and *Staphylococcus saprophyticus*. In asymptomatic urinary tract infection, the number of significant bacteria to support UTI diagnosis is 100,000 mL⁻¹ urine.

Appropriate therapy should be given as early as possible to prevent progression of the infection, such as development of pyelonephritis due to ascending spread or sepsis due to hematogenous spread. Rational antimicrobial treatment should be based on the pattern of the causative microbes. Selection of antimicrobial therapy in outpatient will be different from hospitalized patients, because the pattern of the causative microbes is also different. Increased risk factors and exposure to antibiotics in hospitalized patients, can lead to more varied pattern of the causative microbes, that it is necessary to understand the difference of the causative microbial pattern in female out- and hospitalized patient.

MATERIALS AND METHODS

The Pattern of UTI Causative Microbes in Female Outpatients. Urine was obtained in 2015 from

female outpatients in six Community Health Centers in Jakarta, including Makassar, Pulogadung, Cakung, Pasar Rebo, Duren Sawit, and Kramat Jati Community Health Centers. The subjects were pregnant women who get antenatal care in the six Community Health Centers. The urine was cultured at the Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Indonesia. Urine samples used in this study were midstream urine from women with no risk factor. Before culturing, urine sample was mixed well. Urine was taken using sterile calibrated loop with 0.001 mL size and streaked on blood and McConkey agar plates, followed by incubation at 35-37 °C, for 18-24 h. Only urine samples containing microbes at $\geq 100,000$ cfu mL⁻¹ urine were used for bacterial identification.

The Pattern of UTI Causative Microbes in Female Hospitalized Patients. The information of causative microbial pattern was obtained as secondary data from the Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Indonesia. Culture of midstream urine from female patients hospitalized in several hospitals in Jakarta were analyzed. Only urine samples containing microbes at $\geq 100,000$ cfu mL⁻¹ of urine used for bacterial identification.

Identification of Uropathogen. Identification of the UTI-causing microorganisms from both clinical specimens were done using VITEK 2 compact system® (bioMérieux). Colonies of a pure culture were taken and placed in a test tube containing 3.0 mL sterile saline (0.45%-0.50% NaCl, pH 4.5-7.0) to make a suspension. Suspension turbidity was adjusted to 0.5 McFarland, which was comparable to 1.5×10^8 cfu mL⁻¹. Test tube containing the microorganism suspension was placed into a special rack (cassette) and identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. Rack or cassette containing test tubes and the identification card was inserted into the machine. The filled cassette was placed manually into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the microorganism suspension was forced through the transfer tube into micro-channels that fill all the test wells. The cassette was incubated at 35.5 ± 1.0 °C. Each card was removed from the carousel incubator once every 15 min, transported to the optical system for reaction readings, and then returned to the incubator until the next reading. The identification results will appear on the monitor screen after 3-7 h. The databases of the VITEK 2 identification machine were constructed with large strain sets of well-characterized

microorganisms tested under various culture conditions. Each of the composite values was compared to the others to determine if the data are sufficiently unique or close to one or more of the other database taxa. If a unique identification pattern was not recognized, a list of possible organisms was given, or the strain was determined to be outside the scope of the database. An unknown biopattern was compared to the database of reactions for each taxon, and a numerical probability calculation was performed. Various qualitative levels of identification are assigned based on the numerical probability calculation.

Bacterial identification system used two types of Vitek card, GN card for identification of Gram-negative bacilli and GP card for identification of Gram-positive (primarily cocci). The YST card was used for identification of yeast-like microorganisms.

The data in this study was analyzed by SPSS 20.0 for windows, using Chi-Square method.

RESULTS

The Pattern of UTI Causative Microbes in Female Outpatients. There were 27 of 317 urine samples from the female outpatients contained bacteria at $\geq 100,000$ cfu mL⁻¹. Two of the samples indicated double infection (the presence of two bacteria in an individual). Therefore, it was found 29 microorganisms pattern in this study (Table 1).

Gram-negative bacteria was the most common cause of UTI found in female outpatient (found in 22 cases, (75.9%), while only 7 cases (24.1%) was caused by Gram-positive bacteria found. *Klebsiella pneumoniae*, a Gram-negative bacteria, was found as the most common cause of UTI in female outpatient in this study (found in 31% cases), while *Escherichia coli*, which is also Gram-negative, was found as the second most common cause of UTI in female outpatients (24.1%).

Distribution pattern of the causative microbes by age group can be seen in Table 2. In the age group 16-20 years, four species of bacteria were found, *Klebsiella pneumoniae*, *Enterococcus cloacae*, *Staphylococcus hemolyticus*, and *Alcaligenes faecalis*. However, these four microbes were found at equal frequency. In the age groups 21-30 and 31-40 years, *Escherichia coli* was the most common UTI-causing microbe.

Distribution pattern of the causative microbes by trimester of pregnancy can be seen in Table 3. In the first trimester of pregnancy, three species of bacteria were found, *Acinetobacter baumannii*, *Streptococcus*

agalactiae, and *Streptococcus viridans*. There was no specific pattern in the frequency of occurrence of the three UTI-causing microorganisms in the first semester of pregnancy. In the second trimester, the most commonly found causative agent was *Escherichia coli*, whereas in the third trimester of pregnancy, *Escherichia coli* and *Klebsiella pneumoniae* were the two most common causative bacteria found.

The Pattern of UTI Causative Microbes in Female Hospitalized Patients. Of the 114 urine samples from female hospitalized patients, 29 of them showed significant positive culture results (containing microbes at $\geq 100,000$ cfu mL⁻¹ of urine). The most common microbial cause of UTI in hospitalized females were Gram-negative bacteria (found in 55.2% cases), followed by fungi (24.1% cases), and Gram-positive bacteria (20.6% cases). Among Gram-negative bacteria, *Escherichia coli* was the most common cause of infection (56.3% cases). However, all Gram-positive bacteria were found at equal frequency. *Candida* sp. was the only fungus found as a causative agent.

Comparison of the UTI Causative Microbes in Female Out- and Hospitalized Patients. Microorganisms pattern of Gram-negative as the most common cause of UTI in female outpatient and hospitalized showed the same results of chi-square analysis (Table 5). There were 75.9% UTI in outpatients and 55.2% in hospitalized patients caused by Gram-negative bacteria.

In this study, *Klebsiella pneumoniae*, a Gram-negative bacteria, was found as the most common cause of UTI in outpatients (31%) and the second most common cause in hospitalized patients (6.8%). *Escherichia coli* is the most common UTI causative bacteria in women. In this study, however, *E. coli* was found as the most common cause of UTI in hospitalized patients (31%), while in outpatient *E. coli* was the second most common cause (24.1%).

Gram-positive microorganisms caused 24.1% UTI in outpatients and the 48.3% in hospitalized patients. *Candida* caused 24.1% (7/29) UTI in inpatient, however, no fungus was found in outpatient (Table 7).

DISCUSSION

The Pattern of UTI Causative Microbes in Female Outpatients. Gram-negative bacteria was the most common cause of UTI in female outpatients in this study. The Enterobacteriaceae found as the main pathogen in the urinary tract might have come from the

Table 1 The pattern of UTI-causing microbes found in female outpatients

Gram	Bacteria	Number of Infected Patients	Percentage
Negative	<i>Klebsiella pneumoniae</i>	9	31%
	<i>Escherichia coli</i>	7	24.1%
	<i>Acinetobacter baumannii</i>	2	6.9%
	<i>Alcaligenes faecalis ssp faecalis</i>	1	3.4%
	<i>Enterobacter cloacae ssp cloacae</i>	1	3.4%
	<i>Pseudomonas stutzeri</i>	1	3.4%
	<i>Stenotrophomonas maltophilia</i>	1	3.4%
Positive	<i>Streptococcus viridans, alpha-hem</i>	2	6.9%
	<i>Leuconostoc mesenteroides ss. cremoris</i>	1	3.4%
	<i>Staphylococcus aureus ss. aureus</i>	1	3.4%
	<i>Staphylococcus haemolyticus</i>	1	3.4%
	<i>Streptococcus agalactiae</i>	1	3.4%
	<i>Streptococcus sanguinis</i>	1	3.4%
Total		29	100%

Table 2 UTI-causing microbes in female outpatient distributed by age

Age (in years)	Microbes	Percentage (Number of Infected Patients)
16-20 (n=4)	<i>Klebsiella pneumonia</i>	25% (1)
	<i>Enterococcus cloacae</i>	25% (1)
	<i>Staphylococcus hemolyticus</i>	25% (1)
	<i>Aliccaligenes faecalis</i>	25% (1)
21-30 (n=10)	<i>Escherichia coli</i>	20% (2)
31-40 (n=6)	<i>Escherichia coli</i>	50% (3)
No data (n=9)	<i>Klebsiella pneumonia</i>	66.7% (6)

Table 3 UTI-causing microbes in female outpatients distributed by trimester of pregnancy

Trimester Pregnancy	Microbes	Percentage (Number of Infected Patients)
I (n=3)	<i>Acinetobacter baumannii</i>	33.3% (1)
	<i>Streptococcus agalacte</i>	33.3% (1)
	<i>Streptococcus viridan</i>	33.3% (1)
II (n=6)	<i>Escherichia coli</i>	50% (3)
III (n=12)	<i>Escherichia coli</i>	25% (3)
	<i>Klebsiella pneumonia</i>	25% (3)
No data (n=8)	<i>Klebsiella pneumonia</i>	75% (6)

Table 4 The composition of UTI-causing microorganisms found in female hospitalized patients

Gram	Bacteria	Number of Infected Patients	Percentage
Negative	<i>Escherichia coli</i>	9	31%
	<i>Klebsiella pneumoniae</i>	2	6.8%
	<i>Alcaligenes faecalis (odorans)</i>	1	3.4%
	<i>Enterococcus faecalis</i>	1	3.4%
	<i>Pseudomonas aeruginosa</i>	1	3.4%
	<i>Stenotrophomonas maltophilia</i>	1	3.4%
	<i>Proteus mirabilis</i>	1	3.4%
	Positive	<i>Aerococcus urinae</i>	1
<i>Leuconostoc mesentroides ss cremoris</i>		1	3.4%
<i>Actinomyces odontolyticus</i>		1	3.4%
<i>Streptococcus agalactiae</i>		1	3.4%
<i>Staphylococcus haemolyticus</i>		1	3.4%
<i>Streptococcus sanguinis</i>		1	3.4%
Fungal <i>Candida sp.</i>		7	24.1%
Total		29	100%

Table 5 Comparison of Gram-Negative and non-Gram-Negative Caused UTI in Female Out- and Hospitalized Patients

	Bacteria Pattern				P
	Gram negative		Non Gram negative		
	n	%	n	%	
Outpatients	22	75.9	7	24.1	0.097
Hospitalized Patients	16	55.2	13	44.8	
Total	38	65.5	20	34.5	

intestinal microbiota. Instead of *E. coli*, this study found that *Klebsiella pneumoniae*, which is also a member of Enterobacteriaceae, to be the most common cause of UTI in female outpatients. This result was similar to a study reported by Rajaratnam *et al.* in India (2013), showing that the most common bacteria causing UTI in female outpatients was *Klebsiella pneumoniae* (50%) and *E. coli* (14.2%).

Klebsiella pneumoniae is a Gram negative bacteria, one of normal microorganisms in human's intestine. Morphology of this bacteria is rod-shape, non-motile, and lactose fermenting. *K. pneumoniae* is a facultative anaerobe, therefore it is able to grow either with or without free oxygen. Capsule as a virulence factor of this bacteria that act as physical barrier to overcome the host's immune response. This capsule surround this

bacteria also protects the cell from desiccation. Although found as normal microorganism, *K. pneumoniae* can progress into bacterial infections, including urinary tract infections.

In the age groups 21-30 and 31-40 years, the most frequently found UTI-causing microorganism in female outpatient in this study was *Escherichia coli*. This bacteria is an enteric bacteria residing in the periurethral introitus of the vagina and can migrate up to the bladder through urethra. The lack of hygiene in the female reproductive tract and sexual activity can lead to the migration. *E. coli* infection in women can also come from the rectum, this is due to the close proximity between the rectum and urethral meatus.

Escherichia coli was also the most common UTI causative microbe in the second and third trimesters of

Table 6 Comparison of Gram Positive and non-Gram Positive Caused UTI in Female Outpatients and Hospitalized Patients

	Bacteria Pattern				P
	Gram positive		Non Gram positive		
	n	%	n	%	
Outpatients	7	24.1	22	75.9	0.753
Hospitalized Patients	6	20.7	23	79.3	
Total	13	22.4	45	77.6	

Table 7 Comparison fungal and non-fungal caused UTI in female Outpatients and hospitalized patients

	Bacteria Pattern				P
	Gram positive		Non Gram positive		
	n	%	n	%	
Outpatients	0	0	29	100	0.01
Hospitalized Patients	7	24.1	22	75.9	
Total	7	12.1	51	87.9	

pregnancy. Although UTI in pregnant women often asymptomatic but it can develop to pyelonephritis. Risk of life-threatening illness such as perinatal and neonatal morbidity can increase because of pyelonephritis. Therefore, all pregnant women should be screened for bacteriuria and subsequently treated with appropriate antibiotics.

The Pattern of UTI Causative Microbes in Female Hospitalized Patients. The most common microorganisms causing UTI in hospitalized females were Gram-negative bacteria (55.2%), of which, *Escherichia coli* constituted 56.3%. This result is similar to the study by Alkhyat *et al.* in Yaman (2013), where it was reported that the most common bacteria causing UTI in hospitalized females was *Escherichia coli* (46.7%).

The emerging resistance of *Escherichia coli* to several antibiotics was a challenge for UTI treatment of hospitalized patients. Evidence-based prevention guidelines is strongly recommended to reduce the morbidity and prevent the dissemination of drug-resistant Gram-negative microorganisms in hospital. The most effective management should be followed by removal of the risk factor such as urinary catheter.

This study showed that 20.6% of the UTI cases found in hospitalized females were caused by Gram-positive bacteria. This is similar to research conducted by Beyene *et al.* (2011), who reported that 19.1% of UTI cases in hospitalized females were caused by Gram-positive bacteria. Although Gram-positive bacteria are fairly uncommon UTI causative agents, investigating the efficacy of treatment is very

important to reduce morbidity caused by UTI in hospitalized patients. The predisposing factors in the urinary tract, such as obstruction, indwelling catheters, surgery and chronic debilitating diseases should be removed.

Fungi was the second most common UTI causing microorganism in hospitalized females in this study (24.2%). This is supported by research conducted by Wilson *et al.* (2004), who reported that the frequency of *Candida*-caused UTI in hospitalized patients ranged between 9.4-15.8%. The use of broad-spectrum antibiotics in hospitalized patients could cause an imbalance of the normal flora of the body. The imbalance condition could cause overgrowth of *Candida* as part of normal flora in gastrointestinal tract that can lead to opportunistic UTI in hospitalized patients.

Comparison of the UTI Causative Microbes in Female Out- and Hospitalized Patients. Gram-negatives were found to be the most common UTI-causing microbes in female out- and hospitalized patients with frequency of occurrence 75.9% and 55.2%, respectively. This result was slightly different from Angami *et al.* (2015), reported that Gram-negative microorganisms caused 46% UTI in outpatients and 61.8% in hospitalized patients in India. This result also showed that enteric Gram-negative microorganisms were the most common cause of UTI in female out- and hospitalized patients. Therefore, empirical therapy against Gram-negative microorganisms can be recommended for UTIs in women.

In this study, *Klebsiella pneumoniae*, a Gram-negative bacteria, was found as the most common cause of UTI in outpatients, while in hospitalized patients it was only the second most common. Study conducted by Tajbakhsh *et al.* (2015) showed that 8.2% of UTI were caused by *Klebsiella pneumoniae* both in outpatients and hospitalized patients. Considering *Klebsiella pneumoniae* has a great potential to be resistant to many antibiotics, strategies for treatment will differ between UTI in outpatients and inpatients. The choice of a specific antibiotic depends on local susceptibility patterns. Uncomplicated cases caused by susceptible strains may be treated orally, while intravenous agents are used only if fever is found.

E.coli, a Gram-negative bacteria, was found as the most common cause of UTI in hospitalized patients in this study, while in outpatients, it was the second most common cause of UTI. Although antibiotics are still the standard treatments for UTIs, some strains of *E. coli*, called extended-spectrum beta-lactamase (ESBL) *E. coli*, are resistant to most drugs. ESBL enzymes are able to hydrolyze most of the beta-lactam antibiotics, including third-generation cephalosporins. In addition, ESBL-*E. coli* (EC) might also express co-resistance to SMX/TMP, fluoroquinolones, and aminoglycosides. Carbapenems are generally considered the drug of choice for intravenous treatment of ESBL-EC UTI. Some ESBL-EC isolates will have *in vitro* susceptibility to piperacillin/tazobactam. However, the use of this antibiotic remains controversial. There are limited oral options for the treatment of ESBL-EC cystitis. An alternative is Fosfomycin, an oral antibiotic agent with broad activity against multi-drug resistant pathogens including ESBL-EC.

There is no significant difference in the frequency of Gram-positive microorganisms in the female outpatients and hospitalized patients. However, there is a striking difference between fungal-caused UTI in female outpatients and hospitalized patients. *Candida* was the only UTI-causing fungus found in female hospitalized patients in this study, while there was no fungal-caused UTI in female outpatients. According to our study, the most common UTI-causing microbes in female outpatients in Jakarta was *Klebsiella pneumoniae* (31%) and *Escherichia coli* (24.1%), whereas in female hospitalized patients, the most commons were *Escherichia coli* (31%), *Candida sp* (24.1%) and *Klebsiella pneumoniae* (6.8%). There was no significant difference between the occurrence of Gram-negative and Gram-positive bacteria in out- and hospitalized patients ($p > 0.05$). However, fungal

infection caused by *Candida sp.* was only found in hospitalized patients ($p = 0.01$). This result proved that the UTI-causing microbes found in hospitalized female patients were more varied in comparison to those found in female outpatients.

The results of this study suggested that periodic surveys are necessary to determine the distribution and pattern of UTI-causing microorganisms and that it would be beneficial as guidance for empirical antimicrobial therapy in UTI patients while waiting for the results of urine culture.

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