Bacterial Pathogens in Urinary Tract Infection and Antibiotic Susceptibility Pattern at a Private Hospital in Jakarta, Indonesia

[1]Noor Andryan Ilsan*, [2]Maulin Inggriani, [3]Nurhikmah
Dept. of Medical Laboratory Technology Stikes Mitra Keluarga Bekasi
Email: noorandryanilsan@gmail.com

Abstract
Urinary Tract Infection (UTI) is the most widespread infectious disease in the world after respiratory tract infection. The incidence of UTI in Indonesia are around 90-100 cases in every 100,000 population per year. UTI is an infection caused by the growth of microorganisms in human urinary tract involving the kidneys, ureters, bladder, and urethra. UTIs are caused by a variety of bacteria, but the 90% incidence of UTI is caused by *E. coli*. The pattern of the bacteria causing UTI and its antibiotic susceptibility plays an important role in the treatment of UTI. The aims of this study were to isolate and identify bacterial pathogenic agents that causing UTI at a private hospital in Jakarta and to evaluate their antibiotic susceptibility pattern. A total of 121 (29,2 %) from 293 urine samples collected from patients with UTI symptoms showed a positive bacterial cultures which is the 66,1 % were from females and 33,9 % were from males patients. The UTI- causing bacteria which found were *E. coli* (65,3 %) and *Klebsiella pneumoniae* (13,2 %). The highest incidence of UTI occured in elder patients (38,8 %). Married patients (89,3%) had a higher incidence of UTI compared with single patients (10,7 %). The antibiotic susceptibility test showed that *E.coli* isolated from UTI patients was most resistant to amoxicillin and ampicilin (96.05 %) and most susceptible to Piperacilin (98.7 %). *KPneumoniae* isolated from UTI patients was most resistant to Ampicillin (90,6 %) and most susceptible to Piperacilin (98.83 %).

Key words: Antibiotic susceptibility, bacterial pattern, *E.coli, Klebsiella pneumoniae*, UTI

Introduction

Urinary Tract Infection (UTI) is the most common infectious disease which come from nosocomial pathogens according to infection site after surgical-site and bloodstream infection (WHO 2002). According to Indonesian Ministry of Health (2005), the incidence of UTI in Indonesia are around 90-100 cases in every 100,000 population per year or approximately 180.000 new cases annually. UTI is an infection caused by the growth of microorganisms in the human urinary tractinvolving the kidneys (pyelonephritis), ureters, bladder (cystisis), and urethra. UTI is one of the most common infections affecting patients from different age groups (Yusuf *et al.* 2015). Some studies have shown that UTIs are common in adult females. One in every five adult female experienced UTI in her life and it is extremely common (Hummers- Pradier et al, 2005; Behzadi et al, 2010). More than 95 % of urinary tract infections are caused by single bacterial species. *E. coli* is the most frequent UTI causing bacteria that lead to an acute infections (Moges & Genetu,2002). *E.coli* has been known for having virulence factors which help it occupy the urinary tract and induce inflammation. Those factors include the presence of pili or antigen K in bacterial capsule, fimbriae, haemolysin and colicin production also the ability to acquire iron (Rushton 1997). UTIs were also caused by a variety of bacteria including *Klebsiella* sp., *Pseudomonas* sp., *Acinotebacter* sp., *Staphylococcus* sp., *Raoultella ornithinolytica*, *Serratia* sp., and *Enterobacter* sp. The relative frequency of pathogens varied depending on age, sex, chaterization and hospitalization (Sefton, 2000). Factors that affect the pathogenesis of UTI i.e. gender, sexual activity, age, obstruction, neurogenic dysfunction of urinary bladder, bacterial virulence factors and genetic factor (Flores-Mireles *et al.* 2015). Treatment of UTI was often based on information determined from the antimicrobial resistance pattern of bacterial urine pathogens (Wilson & Gaido 2004). The prevalence of antimicrobial resistance among bacterial urine pathogen has been increasing worldwide due to extensive misuse of antibiotics in practice (Bonadio et al, 2001, Grude et al, 2001). Theaims of this study were to determine bacterial etiologic agents responsible for urinary tract infection and to evaluate their *in vitro* susceptibility

pattern of antibiotics. This study is important to facilitate the effective treatment and management of patient with symptoms of urinary tract infection in Jakarta especially at a private hospital in Jakarta, from January to June 2017.

Method

Data Collection

The microbiological and antibiotics susceptibility data of this study were obtained from the laboratory records of private hospital in Jakarta, Indonesia. These data were collected from January to June 2017.

Urine Collection, Isolation and identification of Bacterial Pathogen

The urine samples from patients were collected by clean catch midstream method in sterile containers and were brought to the laboratory as soon as possible. A total of 293 urine samples were collected during this study. Bacterial isolation were carried out in Blood Agar (BA) and Mac Conkey Agar (MCA) medium (Oxoid, UK). Urine samples were homogenized using 10 µl disposable calibrated ose (Citotes) then were streaked onto agar plates medium. The plates were incubated at 37° C for 24 h. Colonies that grew on the medium were counted. Specimens with bacterial colony count less than 10^5CFU/ml were interpreted as UTI negative and more than 10^5 CFU/ml as UTI positive (Vandepitte et a,. 2002). The Gram negative bacteria isolated from urine samples in this study were identified using API 20E (Biomerieux, USA) biochemical test for testing. Meanwhile Mannitol Salt Agar (MSA) medium (Oxoid, UK), oxidase, catalase and coagulase were used for identification of Gram positive bacteria. The bacterial identification and incidence rate were grouped based on age, gender and marital status.

Antibiotic Susceptibility Test

Antibiotic susceptibility test was carried out on Mueller Hinton agar (Oxoid, UK) using disk diffusion (Kirby Bauer's) method according to the Clinical and Laboratories Standards Institute (CLSI 2014) guidelines using the following 37 antimicrobial agents: Amikacin (30 µg), Amoxicillin (25 µg), Amoxillin Clavulanic Acid (30 µg), Ampicillin (10 µg), Ampicillin Sulbactam (20 µg), Aztreonam (30 µg), Cefadroxile (30 µg), Cefalotin (30 µg), Cefepime (30 µg),
Cefixime (5 µg), Cefoperazone Sulbactam (30 µg), Cefotaxime (30 µg), Cefpirome (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefuroxime (30 µg), Cephalexin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5µg), Cotrimoxazole (25 µg), Doripenem (10 µg), Doxycycline (30 µg), Fosfomycin (50µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Meropenem (10 µg),Moxifloxacin (5 µg), Nalidixic Acid (30 µg), Neomycin (30 µg), Netilmicin (30 µg), Nitrofurantion (300 µg), Norfloxacin (10 µg), Ofloxacin (5 µg), Pipemidic Acid (20 µg), Piperacillin (110 µg), and Tigecycline (15 µg).

Results

A total of 293 samples from suspected UTI patients were collected and 121 samples showed positive UTI. Among the patients that showed positive UTI, 80 (66.1 %) samples belonged to female and 41 (33.9 %) samples belonged to male patients (Table 1). According to the Table 2, results of this study also showed that the predominant isolate was *E. coli* (65.3 %) followed by *Klebsiella pneumoniae* (13.2 %) which was the second most prevalent bacterial pathogen of UTI. Eleven types of other bacterial species have been found and isolated i.e. *Klebsiella oxytoca*, *Proteus mirabilis*, *Acinetobacter baumanii*, *Raoultella ornithinolytica*, *Serratia marcescens*, *Serratia odorifera*, *Pseudomonas flourescens* and *Pseudomonas aerogenes* (Table 1).

Table 1. Pathogenic bacteria in UTI patients and the incidence percentage based on gender

Bacterial pathogen	Male (%)	Female (%)	Total (%)
Eschericia coli	22(17.6)	57(47.7)	79(65.3)
Klebsiella pneumonia	6 (5)	10 (8.2)	16 (13.2)
Klebsiella oxytoca	6 (5)	1 (0.8)	7(5.8)
Proteus mirabilis	2 (1.65)	2 (1.65)	4(3.3)
Acinetobacter baumanii	0	1(0.8)	1(0.8)
Raoultella ornithinolytica	1(0.8)	3(2.5)	4(3.3)
Serratia marcescens	0	1(0.8)	1(0.8)
Serratia odorifera	0	1 (0.8)	1(0.8)
Pseudomonas fluorescens	1(0.8)	0	1(0.8)
Pseudomonas aerogenes	1(0.8)	1(0.8)	2(1.7)
Enterobacter aerogenes	0	1(0.8)	1(0.8)
Enterobacter cloacae	2(1.7)	0	2(1.7)
Staphylococcus aureus	0	1(0.8)	1 (0.8)
Staphylococcus epidermis	0	1(0.8)	1 (0.8)
Total	41(33.9)	80(66.1)	121 (100)

According to Table 2, the highest incidence of UTI occured in elderly patients (> 66 y.o) i.e. 38.8 %. According to Table 3, married patients (89.3 %) had a higher incidence of UTI compared to single patients (10.7 %).

Table 2. Incidence of UTI based on Age

Age	Frequency (%)
0-5	9(7.4)
6-10	2(1.7)
11-16	2(1.7)
17-25	1(0.8)
26-35	6 (5)
36-45	14(11.6)
46-55	21(17.4)
56-65	19(15.7)
>66	47(38.8)
Total	121 (100)

Table 3. Incidence of UTI based on marital status

Marital status	Frequency (%)
Married patient	108 (89.
Single patient	13 (10.7)
Total	121 (100)

The antibiotic susceptibility test result of *E. coli* isolated from UTI patients showed that they were most resistant to amoxicillin and ampicilin (96.05 %) followed by Nalidixic acid (55.26 %) on the other hand *K. pneumoniae* was most resistant to Ampicillin (90.57 %) followed by Ciprofloxacin (90.09 %) and Moxifloxacin (89.67 %). *E. coli* isolated from UTI patients was most susceptible to Piperacilin (98.7 %) followed by Cefoperazone sulbactam, Doripenem and imipenem (98.68 %) meanwhile *K. Pneumoniae* was most susceptible to Piperacilin (98.83 %) followed by Nitrofurantoin (97.44 %) and Cefoperazone sulbactam (95.8 %) (Table 4).

Table 4. Antibiotic susceptibility pattern of *E. coli* and *K. pneumoniae*

No	Antibiotic	Concentration	Resistant (100%) *E.Coli*	*K.Pneumoniae*
1	Amikacin	30	3.90	11.49
2	Amoxicillin	25	96.05	79.35
3	Amoxillin Clavulanic Acid	30	29.33	49.44
4	Ampicilin	10	96.05	90.57
5	Ampicilin Sublactam	20	31.08	60.85
6	Aztreonam	30	5.41	15.27
7	Cefadroxile	30	34.29	53.33
8	Cefalotin	30	52.63	63.69
9	Cefepime	30	3.51	10.47
10	Cefixime	5	28.57	85.11
11	Cefoperazone Sulbactam	30	1.32	4.20
12	Cefotaxime	30	21.05	41.24
13	Cefpirome	30	14.67	32.84
14	Ceftazidime	30	2.63	8.06
15	Ceftriaxone	30	16.00	34.78
16	Cefuroxime	30	28.57	48.78
17	Cephalexin	30	37.84	55.78

18	Chloramphenicol	30	42.11	58.39
19	Ciprofloxacin	5	45.45	90.09
20	Cotrimoxazole	25	35.06	58.38
21	Doripenem	10	1.32	11.63
22	Doxycycline	30	41.89	58.27
23	Fosfomycin	50	6.49	11.49
24	Gentamicin	10	19.74	66.37
25	Imipenem	10	1.32	11.63
26	Levofloxacin	5	41.56	89.26
27	Meropenem	10	2.63	20.83
28	Moxifloxacin	5	43.42	89.67
29	Nalidixic Acid	30	55.26	64.81
30	Neomycin	30	23.29	43.70
31	Netilmicin	30	10.53	25.97
32	Nitrofurantion	300	7.89	2.56
33	Norfloxacin	10	46.67	82.35
34	Ofloxacin	5	43.42	89.67
35	Pipemidic Acid	20	44.74	69.11
36	Piperacillin	110	1.30	1.17
37	Tigecycline	15	2.63	14.93

Discussion

This study was conducted to evalute the distribution of bacterial pathogen species isolated from patients with UTI at a private hospital in Jakarta, Jakarta, Indonesia and their antibiotic susceptibility pattern. Furthermore, it describes the relationship between age, gender, marital status and isolated bacterial pathogens. In this study, 293 samples from clinically suspected UTI patients were collected and 121 samples showed positive UTI. The incidence of UTI were higher and more commonly occured in female (80patients/66.1 %) than male (41 patients/33.9 %) patients. The higher frequency in female was due to anatomic and physical factors related to female urinary tract (Al Sweih et al, 2005). Around 25-30% of females between 20-40 years will experience UTI. The incidence of UTI infection in females increases directly with sexual activity and child-bearing (Wilma, 2002). Females are at risk of developing UTI because of their short urethra and certain behavioral factors such as sexual activity and the use of diaphragms and spermicides which promote coliform bacteria colonization in the periurethral area.

The rates of infection are higher in post menopausal females because of bladder or uterine prolapse causing incomplete obstruction of bladder or difficulty in emptying bladder. Females with estrogen deficiency will undergo changes in her vaginal microflora such as Lactobacilli

which allows an aerobes Gram negative bacteria like *E. coli* to colonize the periurethral (Litza & Brill 2010, Hotchandani & Aggarwal, 2012). The highest incidence of UTI occured in elderly patients. The frequency of UTI incidence leading to hospitalization also increases with age (Foxman et al, 2001). The elderly men will experience structural and functional abnormalities of the urinary tract. The most common is prostatic hyperplasia, which can cause obstruction and leading to UTI (Naber et al, 2008). Furthermore, the elderly are often have a high risk to diabetes mellitus, that will also increase the susceptibility to UTI (Saint *et al.* 2006). The incidence of UTI are higher in postmenopausal females as explained earlier. Married patients (89,3 %) had a higher incidence of UTI compared to single patients (10,7 %).

The incidence of infection in females increases directly with sexual activity and child-bearing (Wilma, 2002). Sexual intercourse ≥ 3 times in a week led to higher incidence of UTI. This association had been reported for cystitis (Amiri *et al.* 2009). The mechanical action of sexual intercourse may facilitate *E. coli* strains to enter the urethra and bladder. Also sexual intercouse can change the normal vaginal flora such as *Lactobacillus* and facilitate *E. coli* colonizing the vagina (Gupta, 1998). Uropathogenic *E. coli* strains have important role for sexual transmission (Brown & Foxman, 2000). Some studies reported that *E. coli* was the most common bacterial pathogen causing UTI (Flores-Mireles *et al.* 2015) followed by *Klebsiella pneumoniae* (Al-Mijalli, 2017).

In this study, *E. coli* and *K. pneumoniae* were most resistant to ampicillin with 96,05 %and 90,57 % resistant percentage respectively. Other studies also reported that ampicillin did not inhibit the growth of *E. coli* (Cunha *et al.* 2016, Indonesian Ministry of Health, 2005). Ampicillin and amoxillin belong to β-lactam antibiotic class which is the oldest and most commonly used class of antibiotic. The extensive misuse and excess usage of antibiotics from β- lactam class induced bacteria to became resistant especially *E. coli* (Hilbert, 2011). Based on a long experienced usage of antibiotics, the therapeutic options for UTI have been developed. The treatment of UTI according to the guidelines of the European Association of Urology (EAU) included fosfomycin and nitrofurantion as first line therapy. As an alternative therapy, Cefpodoxime (Ceftriaxone), Cotrimoxazole and Trimethoprim were possible options which had resistance rate less than 20% (Grabe et al, 2014; Wiedemann et al, 2014).

The results of this study showed that *E. coli* was quite sensitive to Fosfomycin (93.51 %), Nitrofurantoin (92.11 %) and Ceftriaxone (84 %) which is less than 20% resistant. Those results were in line with the result of Grabe et al (2014) and Wiedemann et al (2014) studies. *E. coli* also had a low resistance to Piperacillin (1.3 %) and Imipenem (1.32 %). Those result were in line with previous study which stated that all bacterial UTI had a low or even no resistance to Piperacillin (Sifuentes-Osornio et al, 1996). Meropenem and Imipenem produced satisfactory clinical and bacteriologic response in 90-99 % cases study in vivo with UTI patients (Cox et al, 1995).

Conclusion

Females had a higher incidence of Urinary tract infections than males. Elderly had a higher prevalence of UTI than younger people. Married people had a higher incidence of UTI than single people. *E. coli* is the most predominant bacteria causing UTI followed by *Klebsiella pneumoniae*. *E. coli* and *K. pneumoniae* were most resistant to Ampicillin and Amoxicillin. On the other hand, they were most sensitive to Piperacillin, Doripenem and Imipenem.

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