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Original Research Article

Bacteriological Profile of Urinary Tract Infections among Postmenopausal Women Visiting Alka Hospital, Lalitpur, Nepal

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Abstract:

Urinary tract infection (UTI) is one of the most common diseases encountered worldwide and is a major public health problem in terms of morbidity and financial costs. A cross-sectional study was conducted in Alka Hospital, Lalitpur, Nepal from February to July, 2014. A total of 353 midstream urine samples were collected from postmenopausal women visiting Alka Hospital, Lalitpur, Nepal. The samples were examined by microscopically and culture methods. The isolated organisms were identified by conventional microbiological methods. Antibiotic susceptibility test was performed by modified Kirby-Bauer disc diffusion method according to CLSI (2011) guidelines. Among 353 urine samples processed, 32 % (113) showed significant bacteriuria. Out of 113 bacterial isolates,the prevalence of gram negative bacteria was 97.3% (110) while that of gram positive was 2.7% (3). The most predominating organisms causing UTI were *Escherichia coli* (84.9%) followed by *Klebsiella pneumoniae* (5.3%), *Enterococcus faecalis* (1.7%), *Providencia* spp. (1.7%), *Klebsiella oxytoca* (1.7%), *Proteus mirabilis* (1.7%), *Proteus vulgaris* (0.9%), *Citrobacter freundii* (0.9%) and *Staphylococcus aureus* (0.9%). Antibiotic susceptibility tests of the isolates showed that, most of the gram negative bacterial isolates were sensitive to Piperacillin+Tazobactam followed by Amikacin, Imipenem and Nitrofurantoin, while they were resistant to Amoxicillin. All gram positive isolates were sensitive to Amoxicillin and resistant to Gentamycin. This study showed that higher aged postmenopausal women (>80 years) were at higher risk for UTI than those of lower aged ones (<80 years).

Keywords: UTI, Postmenopausal, Antibiotic susceptibility test.

Introduction

Urinary tract infection (UTI) is the second most common infection after respiratory infection encountered in medical practice among the community and hospitalized patients of all age groups (Kumari et al, 2005; Manikandan et al, 2011).

UTI describes a condition in which microorganisms are established and multiplied within the urinary tract. It is most often due to bacteria (95%), but may also include fungal and viral infection (Cattell, 1996). UTI encompasses a spectrum of clinical entities ranging inseverity from asymptomatic infection to acute cystitis, prostatitis, pyelonephritis and urethritis (Fowler, 1986; Gluisier, 1991).

Symptomatic bacteriuria (SB) is a condition of presence of 10^2 or more organisms per mL of urine with pyuria, 10^5 or high number of other pathogens per mL of urine or any growth of organisms from a suprapubic aspirate of urine (MacLean, 2001). Symptoms of SB includes dysuria, frequent urination, lower back pain, urgency and fever (Cheesbrough, 2000)

Asymptomic bacteriuria (ASB) is commonly defined as the presence of more than 10^5 organisms per mL of urine sample in the absence of declared symptoms and WBCs in excess of 10^4 cells per mL (>10 cells/HPF) of urine (the condition of significant pyuria). Untreated ASB is the risk factor for acute cystitis (40%) and pyelonephritis (25-

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30%) in pregnancy. These cases account for 70% of all cases of symptomatic UTI among unscreened pregnant women (Johnson et al., 2012). Carelessness to ASB leads serious complications like renal failure.UTI have the greater likelihood of infection with the multidrug resistant organisms and also have a greater risk of treatment failure (Puri et al, 2006).

Uncomplicated UTIs typically occur in the healthy non-pregnant adult woman, while complicated UTIs (cUTIs) may occur in both genders and all age groups which are frequently associated with either structural or functional urinary tract abnormalities. The largest group of patients with UTI is adult women (Faruqui and Shetiya, 2013).
Natural menopause is the permanent cessation of menstruation resulting from the loss of ovarian follicular activity or follicle depletion (low estrogen and progesterone). Menopause is declared when women who have not had a spontaneous menstrual period for 1 year and are classified as postmenopausal (Mitchel et al, 2000). Menopause occurs most often between the ages of 50-52 years, with 95% of women having final menstrual period between ages 44-56 years (McKnight et al., 2011). Rates of infection are high in postmenopausal women, because of bladder or uterine prolapse causing incomplete bladder emptying; loss of estrogen with attendant changes in vaginal flora, loss of *Lactobacilli*, which allows to urethral colonization with gram negative aerobes, such as *E. coli* (Chaudhuri et al, 2008).

The urinary tract infection in postmenopausal women is characterized by flank pain, but less likely to report frequency, dysuria and hematuria as in young women. Urinary incontinence is very common in postmenopausal women with estimated prevalence of 14% among 60 to 64 year-old women and 25% among 80 to 86 year old women (Foxman et al., 1997).

This study was designed to identify the causative pathogens responsible for UTI among postmenopausal women attending Alka Hospital in order to diagnose, treat and prevent urinary tract infection.

Materials & Methods

A cross-sectional study was conducted in microbiology laboratory of Alka Hospital, Lalitpur, Nepal from February to July, 2014. In this study, a total of 353 midstream urine samples were collected from postmenopausal women visiting the hospital. The samples were processed immediately for routine examination, culture and antibiotic susceptibility pattern.

Urine sample collection and laboratory analysis

Each suspected postmenopausal women was given a dry, sterile and wide necked leak-proof container for the collection of 10-20 mL of the clean catch mid-stream urine (CC-MSU). Patients were well instructed for the collection of CC-MSU. The urine specimen obtained was observed macroscopically for its color, clarity and turbidity. About 5 mL of urine sample was centrifuged at 3000 rpm for 5 min and the sediment obtained was examined microscopically at high power magnification (40X) for the presence of pus, red blood cells, epithelial cells, casts, crystals, and bacteria. Pus cells ≥5/HPF was considered significant for infection. The number of WBC and RBC were estimated as number per HPF (Chaudhary et al, (2017).

Culture of urine specimens and antibiotic susceptibility test

Cultures of each uncentrifuged urine specimen were done on 5% sheep Blood agar (BA) and Mac-Conkey agar (MA) using semi-quantitative culture method (Cheesbrough, 2000). Urine sample (0.001 mL) was streaked onto the culture media using calibrated loop. The urine samples were thoroughly mixed before inoculating into the agar media. The inoculated BA and MA plates were incubated at 37 ^{0}C for overnight in an inverted position. Identification of significant isolates was done by using standard microbiological techniques as described in Bergey's Manual. After gram staining, biochemical tests were performed for the identification of the genera and species of the organisms. After identification, the antibiotic susceptibility test of the isolates was done by modified Kirby-Baur disk diffusion method as recommended by Clinical Laboratory Standards Institute using Mullen Hilton Agar (MHA) (CLSI/NCCLS, 2005).

Results and Discussion

Pattern of culture result

Out of 353 midstream urine samples, 113 (32.0%) showed significant growth and 240 showed no growth.

Age group and occurrence of UTI among postmenopausal women

In this study, the age of the patients ranges from 50 years to higher than 80 years. The highest number of patients

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(162) belonged to the age group 50-60 and lowest (15) among ages higher than 80 years. However, the case finding rate was 7 (46.6%) among 15 patients of higher than 80 years, while 41 (25.3%) were among 162 patients of 50-60 years. The finding was statistically non-significant (P=0. 06 at 95% CI) (Table 1).

Table 1: Age group and occurrence of UTI among postmenopausal women

Age group (years)	Populations	Positive Number
50-60	162	41 (25.3%)
61-70	105	37 (35.2%)
71-80	71	28 (39.4%)
More than 80	15	7 (46.6%)
Total	353	113

Till these days, various studies have shown higher prevalence of UTI in female than male (Amin et al., 2009). According to Oluremi et al., (2011) and Adedeji et al., (2009), sexual activity has been reported to increase the prevalence of UTI in females.

Because of decrement of the estrogen level from 120 ng/L to 18 ng/L during the menopausal transition, the vaginal canal shrinks in length and diameter and has less glycogen to maintain moisture and suppleness (Raz and Stamm, 1993) which reduces the chance of UTI. High age group postmenopausal females were more vulnerable for infection of UTI than those of low age group.

Physical examination of midstream urine of postmenopausal women

The culture positive rate increased with increase in urine turbidity. Culture positive rate decreased in slightly cloudy urine and no growth was seen in clear urine samples **(Table 2).**

Table 2: Physical examination of midstream urine of postmenopausal women

Appearance	Sample	Negative N	Positive N
Cloudy or Turbid	149	45(30.2%)	104(69.8%)
Slightly Cloudy	87	78(89.7%)	9(10.3%)
Clear	117	117(100%)	-
Total	353	240	113

Pyuria verses bacteriuria in postmenopausal women

Out of 353 urine samples, 288 (81.6%) showed few number of pus cells (<10 WBCs/HPF) and among these, 74 (25.7%) gave positive culture results. Similarly, 65 (18.4%) of total samples showed high number of pus cells (>10 WBCs/HPF), among which 39(60%) gave positive culture results. Pyuria is regarded as significant when high number of pus cells are present i.e. more than 10 WBCs/µl (Table 3).

Table 3: Pyuria verses bacteriuria in postmenopausal women

Pus cells No/HPF	No. of sample	Culture negative	Culture positive
<10	288	214(74.3%)	74(25.7%)
>10	65	26(40%)	39(60%)
Total	353	240	113

The false negative results could be due to early urinary tract infection or presence of asymptomatic UTI patients, diabetes and enteric fever or bacterial endocarditis, whereas false positive results (pyruria with negative culture result) could be due to proper use of antibiotics by the patients or presence of bacteria which were unable to grow on the routine culture media (Tamang et al., 2017).

Occurrence of bacteria in different age groups

Out of 113 growth positivity, 9 different bacterial genera were isolated. Among the bacterial isolates from different age groups, *E. coli* (84.9%) was found to be the most predominant organism followed by *Klebsiella pneumoniae* (5.3%), *Enterococcus faecalis* (1.7%) and others (8.0%). However the finding was statistically non-significant (P= 0.07 at 95% CI) (Table 4).

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Table 4: Occurrence of bacteria in different age groups

Organisms isolated	Age group (years)				Total
	50-60	61-70	71-80	>80	No (%)
Escherichia coli	37	30	23	6	96(84.9%)
Klebsiella pneumonia	2	1	3	-	6 (5.3%)
Enterococcus faecalis	-	2	-	-	2 (1.7%)
Providencia spp.	1	1	-	-	2 (1.7%)
Proteus vulgaris	-	-	1	-	1(0.9)
Klebsiella oxytoca	-	2	-	-	2 (1.7%)
Proteus mirabilis	-	-	1	1	2 (1.7%)
Citrobacter freundii	-	1	-	-	1 (0.9%)
Staphylococcus aureus	1	-	-	-	1 (0.9%)

Higher prevalence of *E. coli* might be due to endogenous source of infection as they are frequent isolates at hospital and community acquired infections. (Chhetri et al., 2011). Similar results were reported by Boyko et al., (2005); Lin et al., (2008); Paryani et al.,(2012) and Tamang et al., (2017). *Klebsiella* species were second common uropathogens (7%) among postmenopausal women. Similar findings were reported by Turan et al., (2008) and Chaudhary et al., (2017).

Tamang et al., (2017) had reported *Enterococcus* spp and *Klebsiella* spp as the second and third most common cause of UTI with isolation rates of 23.1% and 19.2% respectively.

Distribution of gram positive and gram negative bacterial isolates

Out of total 113 bacterial isolates, 110 (97.3%) were gram negative whereas 3 (2.7%) were gram positive bacteria. The most frequent bacteria causing the infection are *E. coli* accounting 75 to 95% of infection (Kolawole et al., 2009**).** Most of the UTI studies showed higher prevalence of gram negative bacteria than gram positive bacteria (Chaudhary et al., 2017**;** Tamang et al., 2017**;** Puri et al., 2006). Other gram negative bacteria include *Klebsiella* spp., *Proteus mirabilis* and *Pseudomonas aeruginosa*, *Citrobacter* spp while *Enterococci* spp and *Staphylococcus* spp are the most frequently implicated gram positive organisms **(**Shankel, 2007). Prevalence of gram negative bacteria could be due to the presence of unique structure which helps for attachment to uroepithelial cells allowing for multiplication and tissue invasion thus resulting invasive infection (Agersew et al., 2012).

Antibiotic susceptibility pattern of bacterial isolates

Amikacin was found to be the most effective drug for 95 (98.9%) *E. coli* isolates. Similarly other effective drugs were Piperacillin plus Tazobactam 89 (92.7%), Nitrofurantoin 79 (82.3%), Gentamycin 78 (81.2%), Imipenem 73 (76.1%) and Cefotaxime 61 (63.5%). The most resistant drug was Amoxicillin against 77 (80.2%) *E. coli* isolates (Table 5).

The most sensitive antibiotics against *K. pneumoniae* were found to be Amikacin (100%), Cefixime (100%), Cefotaxime (100%) and Imipenem (100%) followed by Ofloxacin, Nalidixic acid, Piperacillin plus Tazobactam and Gentamycin (83.3%). The antibiotic Ciprofloxacin and Nitrofurantoin were found to be least sensitive. It was 100% resistant to antibiotic Amoxicillin (Table 6).

Similarly, for *Klebsiella oxytoca*, the sensitivity pattern was found to be the same to that for *Klebsiella pneumoniae* except Ceftriaxone found to be (100%) sensitive. Aktar et al., (2013) reported 60% resistant to Amoxicillin.

In case of *Providencia* species, all of the isolates were found to be sensitive to antibiotics viz. Amikacin (100%), Cefixime (100%), Cefotaxime (100%), Ciprofloxacin (100%), Imipenem (100%) and Piperacillin+Tazobactam (100%). Besides these, antibiotics like Ceftriaxone, Ofloxacin and Nalidixic acid were found to be 50% sensitive. Amoxicillin and Nitrofurantoin were found to be 100% resistant. Similar findings were reported by Mahmoud et al., (2016).

This study showed that the most resistant antibiotic against Proteus mirabilis was found to be Amikacin (100%), Ceftriaxone (100%), Ofloxacin (100%), Nalidixic acid (100%) followed by Cefixime (50%), Cefotaxime (50%) and Ciprofloxacin (50%). It was also found to be Amoxicillin, Imipenem and Nitrofurantoin (100%) sensitive. Whereas in *Proteus vulgaris*, the susceptibility pattern was found to be the same except Cefixime was found to be 100% resistant to the similar study done by Biswas et al., (2014).

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Table 5: Antibiotic susceptibility pattern of bacterial isolates

Organisms isolated	Antibiotics	Antibiotic susceptibility pattern	
		Susceptible isolates (%)	Resistance isolates (%)
E. coli (n=96)	Amikacin	95(98.9%)	1(1.1%)
	Amoxicillin	19(19.8%)	77(80.2%)
	Cefixime	56(58.3%)	40(41.7%)
	Cefotaxime	61(63.5%)	35(36.5%)
	Ceftriaxone	59(61.4%)	37(38.6%)
	Ciprofloxacin	51(53.1%)	45(46.9%)
	Imipenem	73(76.1%)	23(23.9%)
	Ofloxacin	48(50%)	48(50%)
	Nalidixic acid	26(27.1%)	70(72.1%)
	Nitrofurantoin	79(82.3%)	17(17.7%)
	Piperacillin+Tazobactam	89(92.7%)	7(7.3%)
	Gentamicin	78(81.2%)	18(18.8%)
Klebsiella pneumoniae (n=6)	Amikacin	6(100%)	-
	Amoxicillin	-	6(100%)
	Cefixime	6(100%)	-
	Cefotaxime	6(100%)	-
	Ceftriaxone	4(66.7%)	2(33.3%)
	Ciprofloxacin	3(50%)	3(50%)
	Imipenem	6(100%)	-
	Ofloxacin	5(83.3%)	1(16.7%)
	Nalidixic acid	5(83.3%)	1(16.7%)
	Nitrofurantoin	2(33.3%)	4(66.7%)
	Piperacillin+Tazobactam	5(83.3%)	1(16.7%)
	Gentamicin	5(83.3%)	1(16.7%)
Enterococcus faecalis (n=2)	Amikacin	1(50%)	1(50%)
	Amoxicillin	2(100%)	-
	Cefixime	-	2(100%)
	Cefotaxime	-	2(100%)
	Ceftriaxone	-	2(100%)
	Ciprofloxacin	-	2(100%)
	Imipenem	-	2(100%)
	Ofloxacin	-	2(100%)
	Nalidixic acid	1(50%)	1(50%)
	Nitrofurantoin	1(50%)	1(50%)
	Piperacillin+Tazobactam	2(100%)	-
	Gentamicin	-	2(100%)

This study showed that the least number of *Citrobacter freundii* were isolated from positive urine culture. *Citrobacter freundii* was 100% resistant to both antibiotics Amoxicillin and Nalidixic acid and 100% sensitive to most of antibiotics like Piperacillin +Tazobactam, Nitrofurantoin, Nalidixic acid, Ofloxacin, Imipenem, Ciprofloxacin, Amikacin, Cefixime and Cefotaxime.

The gram positive bacteria *Staphylococcus aureus* showed 100% sensitive to Amoxicillin, Cefixime, Ceftriaxone and Ciprofloxacin and 100% resistant to the remaining antibiotics (Amikacin, Imipenem and Gentamycin). In case of *Enterococcus faecalis* most of the antibiotics were 100% resistant like, Cefixime, Cefotaxime, Ceftriaxone, Ciprofloxacin, Imipenem and Gentamycin. Besides these, it was found to be sensitive to Amikacin (100%) and Amoxicillin (100%). similar findings were reported by Biswas et al., (2014).

The regional variation of resistance to antibiotics may be explained in part by different antibiotic practices. The

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influence of excessive and inappropriate use of the antibiotic is responsible for the development of antibiotic resistant.

The treatment of bacterial UTIs requires the use of an effective antibiotic, at a dose sufficient to achieve a therapeutic concentration in the UTI. Antibiotics are considered the most important advancement in the history of modern medicine, but the excessive and inappropriate use of antibiotics is thought to be one of the most important factors influencing the increased prevalence of antibiotic resistance. A high percentage of resistance (77.9%) to the Amoxicillin was seen in our study which has been found worldwide (Hooton, 2001). Almost all of the isolates were found to be sensitive to Amikacin (94.7%) and Piperacillin+Tazobactam (92%), which is similar to the report given by Akter et al., (2016).

Amikacin and Piperacillin + Tazobactam are an option for therapy for UTIs. Nearly an equal percentage of sensitive (50.5%) and resistant (49.5%), to the Ofloxacin was seen in this study, so it may or may not be an application for therapy to UTIs has been considered. The resistance pattern of *E. coli, Providencia* spp., Proteus mirabilis and *Klebsiella pneumoniae* to different antibiotics is increasing which emphasizes the need for judicious use of antibiotics whereas *Staphylococcus aureus* and *Enterococcus faecalis* exhibited sensitive to Amoxicillin, Cefixime and Ciprofloxacin. So these may be the drugs of choice for the treatment of UTI caused by Gram positive bacteria.

Conclusions

The prevalence of UTI was highest among the postmenopausal women aged more than 80 years and lowest among those aged between 71-80 years. The infection was mostly caused by bacteria, especially the gram negative. *E. coli* had the highest prevalence among postmenopausal women. Antibiotic susceptibility tests of the isolates showed that, most of the gram negative bacterial isolates were sensitive to Piperacillin + Tazobactam followed by Amikacin, Imipenem and Nitrofurantoin while they were resistant to Amoxicillin. All gram positive isolates were sensitive to Amoxicillin and resistant to Gentamycin. The excessive and inappropriate use of antibiotics is the most important factors influencing the increased prevalence of antibiotic resistance. The overall antibiotic susceptibility testing showed that urinary isolates had increasing resistance to antibiotics. These results suggest for a continual evaluation and regular monitoring of antimicrobial agents for proper treatment.

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