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Original Research Article

Prevalence of Bacterial Uropathogens Among Diabetic Patients Attending Padma Nursing Hospital of Western Nepal

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Abstract

Urinary tract infections (UTIs) are common in patients with diabetes mellitus (DM). The propensity of the infection can vary in different individuals especially when there is underreporting from the patients having a risk of acquiring infections. This study was conducted in Padma Nursing Home, Pokhara, Nepal from April to September, 2014. A total of 200 mid-stream urine samples were collected from diabetic patients visiting the nursing home and processed by standard laboratory procedure. Antibiotic susceptibility test was performed by modified Kirby-Bauer disk diffusion method. About 13% of the patient's urine specimens had shown UTIs. The prevalence of UTI was 18.1% in female and 8.5% in male. Statistically significant association of UTI was found with gender and pyuria. *E. coli* was the most predominating organism (37%) causing UTI followed by *Enterococcus* spp (22.2%) and *Klebsiella* spp. *(*14.8%). Nitrofurantoin and Ciprofloxacin were the most potent drugs.UTIs are more frequent and are likely to have a more complicated course in patients with DM. This study pointed out that higher age patients, female gender and diabetic patients are risk groups for UTI and should be very cautious to so as to prevent it.

Keywords:UTI, diabetes, antibiotic susceptibility test

Introduction

UTI is one of the most common infections encountered in medical practice among the community and hospitalized patients of all ages (Manikandan et al., 2011, Mekki et al., 2010). Symptomatic bacteriuria is defined as the presence of 10^2or more coliform organisms per ML of urine plus pyuria, 10^5 or greater number of other pathogens per ml, or any growth of organisms from a supra pubic aspirate of urine (MacLean, 2001). Asymptomatic bacteriuria (ASB) is commonly defined as the presence of more than 10^5 organism/mL of the urine sample in the absence of declared symptoms and WBCs in excess of 10^4 cells/mL (>10cells/HPF) of urine will indicate significant pyuria. Untreated ASB is a risk factor for acute cystitis (40%) and pyelonephritis (25-30%) in pregnancy. These cases account for 70% of all cases of symptomatic UTI among unscreened pregnant women (Johnson et al., 2012). *E. coli is* most commonly encountered uropathogens covering up to 41.66 % of renal infections. The higher prevalence of *E. coli* may be due to poor hygienic condition of the patients and it is especially higher among females due to contamination of perineum through the faecal flora (Jha et al., 2009).

Diabetes causes several abnormalities of the host defense system that might result in a higher risk of certain infections, including UTI. These include immunologic impairments such as impaired migration, intracellular killing, phagocytosis, and chemotaxis of polymorphonuclear leukocytes from diabetic patients, and neuropathic complications, such as impaired bladder emptying. In addition, a higher glucose concentration in the urine may create a culture medium for pathogenic microorganisms (Boyko et al., 2005). UTI is a serious problem for people with DM. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism due to insulin deficiency and /or insulin resistance, evolving from interaction of variety of genetic and environmental factors. Clinical laboratory records of cases of diabetes were studied for the spectrum of bacterial isolates causing UTI and their antibiotic susceptibility results were analyzed for recommending suitable therapy and to investigate the association between diabetes and UTI.

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Materials and Methods

The study was conducted in microbiology laboratory of Padma Nursing Home, Pokhara, Nepal from April to September, 2014. In this study, about 200 mid stream urine samples were collected from the patients visiting the hospital. All the samples were collected from diabetic patients and the urine samples were processed immediately after collection for routine examination, culture and antibiotic susceptibility pattern.

Urine sample collection and laboratory analysis

Each patient 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 after its color and turbidity was noted. The number of WBC and RBC were estimated as number per high power field (HPF) i.e. 40 X objective of microscope. Other observations done in microscopic examination were casts, crystals, epithelial cells and bacteria. Albumin, pH and sugar were detected in the urine sample by using uristix. The uristix was dipped into the urine specimen and the change in color in the test area was noted after 30 seconds. The change in color of the test area was compared to that of standard color and the result was interpreted.

Culture of urine specimens and Antibiotic Susceptibility Test

Cultures of each uncentrifuged urine specimens were done on 5% Sheep Blood Agar (BA) and MacConkey Agar (MA) using semi-quantitative culture method (Cheesbrough, 2000). Fixed and known volume (0.001 mL) of urine sample was streaked into the culture media using calibrated loop. The urine samples were thoroughly mixed before inoculating into the agar media. The inoculated MA and BA plates were incubated at 37°C for overnight in an inverted position. Identification of significant isolates was done by using standard microbiological techniques as described in the Bergey's Manual which involves morphological appearance of the colonies, staining reactions and biochemical properties. After gram staining, biochemical tests were performed for the identification of the genera and species of the organisms. After identification, the antibiotic susceptibility testing of the isolates was done by modified Kirby-Bauer disk diffusion method as recommended by Clinical Laboratory Standards Institute using Mullen Hinton Agar (MHA) (CLSI/NCCLS, 2005).

Results and Discussion

Among 200 patients, 94 were female and 106 were male. Out of the total 200 patients, 26 (13%) patients had UTI. Proportion of infected female was greater (18.1%) than proportion of infected male (8.5%) and the difference was statistically significant ($p<0.044$).

Among the total of 200 urine samples, 182 (91%) showed few number (up to 10 WBCs/HPF) of pus cells and among these 8.2% (15 samples) gave positive culture results. Similarly 15 (7.5%) of total samples showed moderate number (11-40 WBCs/HPF) of pus cells and among these 60% (9 samples) gave positive culture results. Out of 200 samples, 3 (1.5%) showed many (>40 WBCs/HPF) pus cells and among these 66.7% (2/26 samples) gave positive culture results. The higher number of pus cells in urine to the positive culture results was statistically significant ($p=0.000$) (Table 1). Pyuria is regarded as significant when moderate and many pus cells are present i.e. more than 10 WBCs/µl.

Greater proportion of asymptomatic cases was found than symptomatic cases both in male and female diabetic patients. Out of 17 female UTI cases, 12 (70.6%) showed asymtomatic UTI cases and out of 9 male UTI cases 7 (77.8) showed asymptomatic cases; statistically this difference was insignificant.

Gram negative bacteria accounted for 70.4% and 29.6% was accounted by gram positive bacteria among the significant growth culture in diabetic patients. Among the total bacterial isolates from the urine samples, *E. coli* was the major isolate (37%) followed by *Enterococcus* spp. (Figure 1).

Antibiotic susceptibility pattern of bacterial isolates

Nitrofurantoin, Ciprofloxacin and Amikacin were found to be most effective drugs for 9 (90%) *E. coli* isolates. Similarly other effective drugs were Norfloxacin and Cotrimoxazole both being effective to 7 (70%) *E. coli* isolates. Cent percent sensitivity was shown by isolated strains of *Enterococcus* spp to antibiotics Cefotaxime, Nitrofurantoin, Cotrimoxazole and Ciprofloxacin. Also 83.3% isolates of *Enterococcus* spp showed sensitivity to antibiotics Amikacin and Ofloxacin whereas maximum isolates i.e. 66.7% were resistance to Cefixime and Azithromicin (Table 3).

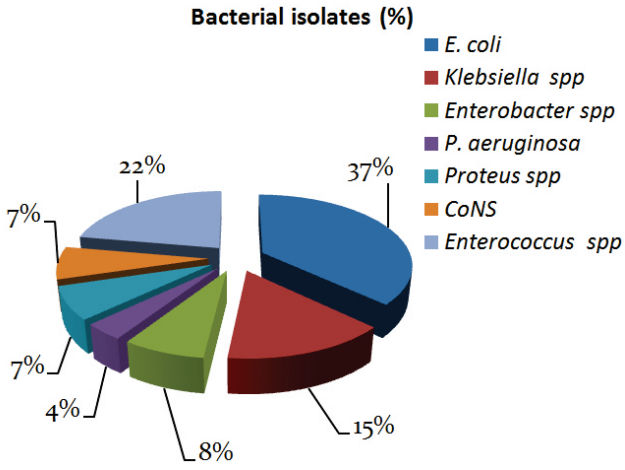

Figure 1: Bacterial isolates among diabetic patients.

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Table 1: Culture positive according to number of Pus cells

Number of pus cells	Culture positive No. (%)	Culture negative No. (%)	Total (%)	p-value
Few	15 (8.2)	167 (91.8)	182 (100)	
Moderate number	9 (60)	6 (40)	15 (100)	0.000
Many	2 (66.7)	1 (33.3)	3 (100)	
Total	26 (13)	174 (87)	200 (100)	

Table 2: Gender wise distribution of symptomatic and asymptomatic UTI cases

UTI cases	Diabetic Patients		p-value
	Female (%)	Male (%)	
Asymtomatic	12 (70.6)	7 (77.8)	
Symtomatic	5 (29.4)	2 (22.2)	0.694
Total	17 (100)	9 (100)	

Table 3: Antibiotic susceptibility pattern of bacterial isolates

Organisms isolated	Antibiotics used	Antibiotic susceptibility pattern	
		Susceptible isolates (%)	Resistance Isolates (%)
E. coli (n=10)	Amikacin	9 (90)	1 (10)
	Azithromicin	6 (60)	4 (40)
	Ciprofloxacin	9 (90)	1 (10)
	Cefixime	5 (50)	5 (50)
	Cotrimoxazole	7 (70)	3 (30)
	Cefotaxime	4 (40)	6 (60)
	Ofloxacin	6 (60)	4 (40)
	NItrofurantoin	9 (90)	1 (10)
	Norfloxacin	7 (70)	3 (30)
Klebsiella spp	Amikacin	4 (100)	0 (0)
	Azithromicin	3 (75)	1 (25)
	Ciprofloxacin	3 (75)	1 (25)
	Cefixime	1 (25)	3 (75)
	Cotrimoxazole	2 (50)	2 (50)
	Gentamicin	4 (100)	0 (0)
	Ofloxacin	2 (50)	2 (50)
	Nalidixic acid	1 (25)	3 (75)
	Nitrofurantoin	4 (100)	0 (0)
*Enterobacter*spp	Amikacin	5 (83.3)	1 (16.7)
	Azithromicin	2 (33.3)	4 (66.7)
	Ciprofloxacin	6 (100)	0 (0)
	Cefixime	2 (33.3)	4 (66.7)
	Cotrimoxazole	6 (100)	0 (0)
	Ofloxacin	5 (83.3)	1 (16.7)
	Nitrofurantoin	6 (100)	0 (0)
	Cefotaxime	6 (100)	0 (0)

In this study, prevalence of UTI in total patients was 13%. Out of 200 urine samples processeed, 94 (47%) were from female, while 106 (53%) were from male. Age group of 41-50 years had maximum request of 26% for urine culture among all diabetic patients. The patients from age group of 21-31 have more chances of exposure to uropathogens, more sexually active, higher cases of pregnancies and high consciousness to health.

Out of 182 cases of insignificant pyuria, 15 gave culture positive and 167 gave culture negative among diabetic patient. Similarly out of 18 cases of significant pyuria, 11 cases were culture positive and 7 were culture negative. Based on this result, the sensitivity and specificity of pyuria as a screening test for urinary tract infection were calculated as 42.3% and 95.98% respectively. Thus it was seen that although the presence of WBCs were specific for significant bacteriuria, the sensitivity was relatively low. In a study carried out in Kathmandu (Shrestha et al., 2004), the sensitivity and specificity of pyuria were found to be 67.3% and 93.8% respectively. 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 (pyuria with negative culture result) could be due to prior use of antibiotics by the patients or presence of bacteria which were unable to grow on the routine

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culture media. Asymptomatic cases in both male and female diabetic patients are more than in symptomatic cases. In this study 71.1% of the diabetic patients had asymptomatic UTI. Sibi et al., (2011) presented that, 68% of the diabetic patients had asymptomatic UTI. Jha et al (2009) observed 9.43% prevalence of UTI among elderly diabetic patients. In case of diabetic female 70.6% (12/17) asymptomatic UTI cases was found and 77.8% (7/9) asymptomatic UTI cases was found in case of diabetic male. In diabetic female, 72.34% asymptomatic UTI cases was found in a study by Ophori et al (2010). Also, in another study done among school students (Frank-Peterside and Wokoma, 2009), asymptomatic bacteriuria in female was 60% and that in male was 40%. Since female are at high risk of UTI than male due to short urethra and prevalence of asymptomatic UTI is also higher in female. So routine screening of UTI should be done in female even if the symptoms are not present. Gram negative bacteria were the major isolates in diabetic patients. The other studies carried out by Jha et al (2005), Cruz et al (2009) and Janifer et al (2009) also concluded Gram negative organisms as major isolates of urine sample.

Out of 7 different bacterial isolates, *E. coli* (37%) was the dominant organism. Adeyeba et al., (2007), ; Jha et al., (2005), Mansour et al., (2009) and Papazafiropoulou et al (2010) also reported *E. coli* as the major UTI causing organism. In our study, *E. coli* was followed by *Enterococcus* spp, and *Klebsiella* spp. In a study by Boyko et al., (2005) the uropathogens isolated did not differ by diabetic status. The source of Gram positive uropathogens especially is other than large bowel such as previous catheterization. *E.coli* is isolated in 90% of Urinary tract infections and strains are characterized by unique virulence determinant, the p pilus (Gal-Gal) (Kot et al., 2010). *Enterococcus* spp (22.2%) was second most common pathogen in our study. Similar reporting was done by Alzohairy and Khadir (2011), Vasquez and Hand (2004) and Manjunath et al., (2011) showing that gram positive bacteria are becoming one of the main uropathogens of UTI. *Enterococcus* often is a problem in complicated UTI in patients with indwelling uretheral Catheters, or patients receiving broad spectrum antibiotics for another infection (Dimitrov et al., 2004). Al Benwen et al (2010) has reported *Streptococcus agalactiae* as the second most common isolate after *E. coli* in their study on 56,506 urine samples. Adedeji and Adbulkadir (2009) reported *Staphylococcus saprophyticus* as the second most common cause of UTI with isolation rate of 23.8%.

Amikacin, Ciprofloxacin and Nitrofurantoin were the most potent drug for the treatment of Gram negative bacteria each being effective for 9 (90%) *E. coli* isolates. This high efficacy of Amikacin was also found by Mutate et al., (2004), Raza et al (2011). Mansour et al., (2009) found the efficacy of Amikacin as 90.5—100%. Nitrofurantoin and Ciprofloxacin was the most effective drug for gram positive organisms. Cent percent sensitivity was shown by isolated strains of *Enterococcus* spp to antibiotics Cefotaxime, Nitrofurantoin, Cotrimoxazole and Ciprofloxacin. Nitrofurantoin, and Ciprofloxacin were the drugs effective to both the isolates of CoNs. Nalidixic acid and Cefotaxime were found non effective to both isolates of CoNS. Nitrofurantion was found most effective drug for both gram positive and gram negative organisms in our study. Nitrofurantoin was also found the most effective antimicrobial in UTI caused by *E. coli* from studies in Nepal (Karki et al., 2004, Sharma et al., 2011, USA (Jamie et al., 2002, (Sahm et al., 2001), Nigeria (Okonko et al., 2009), Iran (Behroozi et al., 2010); Spain (Alos et al., 2004) and Turkey (Eryimaz et al., 2010).

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