

RESEARCH ARTICLE

PATTERN OF BACTERIA CAUSING URINARY TRACT INFECTION AND THEIR ANTIBIOTIC SUSCEPTIBILITY PROFILE IN DIABETIC AND NON-DIABETIC PATIENTS IN LALITPUR, NEPAL- A HOSPITAL BASED STUDY

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ABSTRACT

Background: Urinary tract infection (UTI) is a common problem in diabetic and non-diabetic patients; if it is unrecognized or not treated properly on time can cause considerable morbidity and mortality. The aim of this study is to describe the pattern of bacteria causing UTI in clinically diagnosed diabetic and non-diabetic patients and their antimicrobial susceptibility pattern.

Methods: A hospital laboratory based cross-sectional study was conducted among the diabetic and non-diabetic patients with UTI visiting in Alka Hospital, Lalitpur from September to November 2013. Urine culture, blood sugar test and antibiotics susceptibility test was performed following standard laboratory protocols.

Results: Altogether 1172 midstream urine samples were collected, 330 samples from diabetic and 842 samples from non-diabetic patients. There were 33.6% and 12.0% urine culture positive among diabetic and non-diabetic patients respectively. *E. coli* was found to be the most predominant organism causing UTI in both groups of patients; nitrofurantoin and gentamycin were found most effective against gram negative bacteria. Among the total *E. coli* isolates, there were 60.6% and 57.6% multi drug resistant strain among diabetic and non-diabetic patients with UTI.

Conclusions: Diabetic patients are relatively at higher risk of UTI in comparison to the non-diabetic patients. *E. coli* was the most predominant organism causing UTI. Multi drug resistant strain of *E. coli* in both groups of patients with UTI was a significant problem and it is a serious issue now.

Key words: Antibiotic susceptibility; Bacteria; Diabetes; Multi-drug resistance; Urinary tract infection.

INTRODUCTION

Urinary tract infection (UTI) is a condition in which the urinary tract is infected with uropathogens causing inflammation, which is a common, distressing and occasionally life threatening condition usually requiring urgent treatment. This is one of the most common infections worldwide both in males and females in the community and hospital settings, occurring in all age groups (Kolawole *et al.*, 2009 and Sibi *et al.*, 2011). Diabetes Mellitus (DM) causes several abnormalities of the host defense system that might result in a higher risk of certain infections, including UTI. In addition, a higher glucose concentration in the urine may create a culture medium for pathogenic microorganisms (Boyko *et al.*, 2005). Therefore among the patients with Diabetes if UTI is unrecognized or not treated properly on

time, can cause considerable morbidity and mortality (Patterson and Andriole, 1995). Bacterial resistance is emerging worldwide as a threat to the favorable outcome of common infection in the community and hospital settings (Pitout *et al.*, 2005). Therefore, for the successful management of UTI in both diabetic and non-diabetic patients depends on the proper identification of the bacteria responsible and the selection of effective antibiotics against them. The emergence of resistant bacterial strains in hospitals poses a continued challenge to treat such infections. In many parts of Nepal, there is lack of facilities for urine culture and antimicrobial susceptibility testing, leading to improper diagnosis and irrational antibiotic treatment of UTI. So, inappropriate use of antibiotics is recognized as a significant contributing factor to the spread of bacterial resistance and the development of resistance to antimicrobial agents. This study was aimed to describe the pattern of bacteria causing UTI in clinically diagnosed diabetic and non-diabetic patients and their antimicrobial susceptibility pattern.

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MATERIALS AND METHODS

Study design, setting and ethical consideration: A hospital laboratory based cross-sectional study was carried out in Alka Hospital, Lalitpur, Nepal from September to November 2013. Approval for this study was taken from Kathmandu College of Science and Technology (KCST) and Alka Hospital. The research was conducted according to the principles of the Declaration of Helsinki and informed consent was taken from patient before data collection.

Inclusion and exclusion criteria: Old or newly diagnosed diabetic patients or non-diabetic patients with symptoms of UTI, who gave Mid-stream urine (MSU) sample for routine or culture test and the blood sample for fasting and two-hour postprandial glucose level, were enrolled. Patients with Diabetes and Non-Diabetes were differentiated based on American Diabetes Association (ADA) guideline.⁶ Old diabetic patients with UTI but normal blood glucose levels were excluded. The organism from urine sample showing mixed growth was also excluded from the study.

Sample size, sample collection and laboratory analysis: A total of 1172 MSU and blood samples were collected from the patients visiting the hospital and included in the study. Among the collected samples patients with diabetes were 330 and non-diabetic were 842. The end outcome was to assess UTI causing organism among diabetic and non-diabetic patients. So, the final samples were 111-diabetic, and 101- non-diabetic patients included in final analysis. Cultures were done on Blood agar and Mac-Conkey agar by the Semi-Quantitative method. Sample with more than 10^5 cfu/mL bacteria were considered as positive. Isolation and identification of the organisms were done following standard laboratory protocol as per American Society of Microbiology (ASM) (Isenberg, 2002). Antibiotic susceptibility tests of isolates were performed by Kirby-Bauer disc diffusion method. The antibiotic discs used were amoxicillin, ciprofloxacin, cotrimoxazole, ceftazidime, cefixime, cefotaxime, cephalixin, gentamicin, ofloxacin, nitrofurantoin and vancomycin. Results were interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines (2014). Blood sample was collected twice from the same patient, one sample for fasting blood sugar level and the other for postprandial blood sugar level. Blood sugar was detected by enzymatic method using standard kit and standard protocols as provided by the manufacturer, test was performed by the staffs of Biochemistry Laboratory.

Statistical analysis: Data were entered and statistical analysis was performed using the SPSS version 16.0. Descriptive statistics were used to describe the socio-demographic characteristics. Odds ratio (OR) within 95% confidence interval (CI) was calculated to measure the strength of association, Chi-square test was used for statistical significance and p-value <0.05 was considered significant.

RESULTS

Socio-demographic characteristics: The socio-demographic characteristics of the participants are summarized in Table 1. Among the total 1172 participants, the median age was 32 (minimum-20, maximum-94). The proportion of male vs. female in diabetic and non-diabetic group was 24.3% vs. 75.7% and 14.9% vs. 85.1% respectively. The highest growths were obtained from the samples of patients of age groups 51-70 (45.9%) and 31-50 (34.6%) in diabetic and non-diabetic

patients respectively. In diabetic group, 33.6% (n=111) urine samples was culture positive, whereas in case of non-diabetic group, 12.0% (n=101) was urine culture positive. Out of 111 bacterial isolates in diabetic patients, 105 (94.6%) were found to be Gram negative bacilli and rest 6 (5.4%) were found to be Gram positive cocci whereas in non-diabetic patients, out of 101 bacterial isolates, 97 (96.0%) were Gram negative bacilli and 4 (4.0%) were Gram positive cocci. In both group *E. coli* was the most predominant organism, 89.2% (n=99) in diabetic and 77.2% (n=78) in non-diabetic group. Only 10.8% (n=12) and 22.7% (n=23) were other organisms isolated in diabetic and non-diabetic group respectively.

Pattern of bacterial isolates causing UTI in diabetic and non-diabetic patients: The most organisms isolated from Diabetes were *E. coli* followed by *S. aureus*, *K. pneumoniae* and *Providencia* spp., similarly in Non-Diabetes, the most common organism was *E. coli* followed by *K. pneumoniae*, *S. aureus*, *Providencia* spp. etc.

Antibiotic susceptibility pattern of bacterial isolates: Nitrofurantoin and gentamicin were found as the most effective drug against Gram negative bacteria from both diabetic and non-diabetic patients (table 2). Gram positive isolates (n=6), *S. aureus*, exhibited maximum resistance towards amoxicillin, cephalixin, cotrimoxazole, ciprofloxacin and ofloxacin (100% each). The organisms showed moderate resistance towards ceftazidime and gentamicin (50% each) but susceptible toward vancomycin and gentamicin (50% each). So, vancomycin and gentamicin can be the drug of choice against Gram positive bacteria from diabetic patients. In non-diabetic patients, gentamicin was the drug of choice against Gram positive, *S. aureus* isolates. Also vancomycin, ciprofloxacin and ofloxacin were sensitive against *S. aureus* as shown in table 2. Due to low isolation rate of other isolated organisms, only the antibiotic susceptibility pattern of *E. coli* and *K. pneumoniae* between diabetic and non-diabetic group was shown in table 3.

Association in between different variables with UTI among diabetic and non- diabetic group: Table 4 shows the association of different variables with UTI among diabetic and non- diabetic group. Age more than or equal to 51 years was significantly associated with diabetic patient with UTI (OR=3.09, 95%CI=1.76-5.43, p=0.0001). Diabetic patients with UTI had more urine culture positivity than non-diabetic group of patients (OR=3.71, 95%CI=2.73-5.06, p=<0.0001). Also the association between *E. coli* and UTI in diabetes was significant (OR=2.43, 95%CI=1.13-5.19, p=0.021). In accordance with antibiotics used, Quinolones and β -lactams class of antibiotics were significantly resistant among Diabetic patients with UTI (p<0.05) whereas nitrofurantoin was significantly resistant among non-diabetic patients with UTI (p<0.05).

Multidrug resistance (MDR) pattern of organisms: In our study among 99 *E. coli* isolates, 60.6% (n=60) were identified as MDR strain in diabetic patients; whereas in non-diabetic patients, out of 78 *E. coli* isolates 57.6% (n=45) were found as MDR strain. All *K. pneumoniae* isolates were found as MDR in diabetic whereas 66.6% (n=4) were found as MDR in non-diabetic patients. Likewise, *S. aureus* showed 100% (n=6) MDR in diabetic patients, whereas in non-diabetic patients 75% (n=3) were found as MDR strain. MDR pattern of isolated organisms in non-diabetic patients were shown in table 5.

Table 1. Socio-demographic characteristics N=1172

Characteristics	Diabetic n (%)	Non-diabetic n (%)
Age		
≤ 50	42 (37.8)	66 (65.3)
≥ 51	69 (62.2)	35 (34.7)
Total	111	101
Median Age	32(min-20, max-94)	
Gender		
Male	27(24.3)	15(14.9)
Female	84(75.7)	86(85.1)
Total	111	101
Culture		
Positive	111(33.6)	101(12.0)
Negative	219(66.3)	741(88.0)
Total	330	842
Bacteria		
Gram negative	105(94.6)	97(96.0)
Gram positive	6(5.4)	4(3.9)
Total	111	101
Organisms		
E. coli	99 (89.2)	78 (77.2)
*Others	12 (10.8)	23 (22.7)

*Others - *P. mirabilis*, *K. oxytoca*, *K. pneumoniae*, *M. morgani*, *Acinetobacter* spp., *Providencia* spp., *Citrobacter* spp., *S. aureus*, *Enterobacter* spp. and *P. aeruginosa*

Table 2. Antibiotic susceptibility pattern of gram negative and gram positive bacteria

Antibiotics	Diabetic group (n=105)			Non-diabetic group (n=97)		
	Susceptibility pattern			Susceptibility pattern		
	Res.	Int.	Sen.	Res.	Int.	Sen.
Gram negative bacteria						
Amoxicillin	84 (80.0)	3 (2.8)	18 (17.1)	78(80.4)	2(2.0)	17(17.5)
Cefotaxime	39(37.1)	3 (2.8)	63 (60.0)	27(27.8)	1 (1.0)	69(71.1)
Cefixime	39(37.1)	3 (2.8)	63 (60.0)	30(30.9)	1(1.0)	66(68.0)
Cotrimoxazole	36(34.2)	3 (2.8)	66 (62.8)	42(43.2)	0	55(56.7)
Ciprofloxacin	42(40.0)	0	63 (60.0)	32(32.9)	1(1.0)	64(65.9)
Ofloxacin	48(45.7)	0	57 (54.2)	35(36.0)	0	62(63.9)
Nitrofurantoin	6(5.7)	0	99 (94.3)	17(17.5)	2(2.0)	78(80.4)
Gentamicin	9(8.5)	0	96 (91.4)	10(10.3)	2(2.0)	85(87.6)
Gram positive bacteria						
	Diabetic group (n=6)			Non-diabetic group (n=4)		
Amoxicillin	6 (100)	0	0	3(75.0)	0	1(25.0)
Ceftazidime	3 (50)	3 (50)	0	2(50.0)	1(25.0)	1(25.0)
Cephalexin	6 (100)	0	0	1(25.0)	1(25.0)	2(50.0)
Cotrimoxazole	6 (100)	0	0	2(50.0)	1(25.0)	1(25.0)
Ciprofloxacin	6 (100)	0	0	1(25.0)	0	3(75.0)
Ofloxacin	6 (100)	0	0	1(25.0)	0	3(75.0)
Gentamicin	0	3 (50)	3 (50)	0	0	4(100)
Vancomycin	3 (50)	0	3 (50)	0	1(25.0)	3(75.0)

Data presented were number (%).

Res.- Resistance, Int.- Intermediate, Sen.- Sensitive

Table 3. Antibiotic susceptibility pattern of bacteria

Antibiotic	Susceptibility test of <i>E. coli</i>						Susceptibility test of <i>K. pneumoniae</i>					
	Diabetic (n=99)			Non- diabetic (n=78)			Diabetic (n=3)			Non- diabetic (n=6)		
	Res.	Int.	Sen.	Res.	Int.	Sen.	Res.	Int.	Sen.	Res.	Int.	Sen.
Amoxicillin	81 (81.8)	3 (3.0)	15 (15.1)	64 (82.0)	2 (2.5)	12 (15.3)	3 (100)	0	0	5 (83.3)	0	1 (16.6)
Cefotaxime	39 (39.3)	3 (3.0)	57 (57.5)	23 (29.4)	1	54 (69.2)	0	0	3 (100)	2 (33.3)	0	4 (66.6)
Cefixime	36 (36.3)	3 (3.0)	60 (60.0)	26 (33.3)	1	51 (65.3)	0	0	3 (100)	2 (33.3)	0	4 (66.6)
Cotrimoxazole	33 (33.3)	3 (3.0)	63 (63.6)	32 (41.0)	0	46 (58.9)	0	0	3 (100)	3 (50.0)	0	3 (50.0)
Ciprofloxacin	42 (42.2)	0	57 (57.5)	29 (37.1)	1	48 (61.5)	3 (100)	0	0	1 (16.6)	1 (16.6)	4 (66.6)
Ofloxacin	48 (48.4)	0	51 (51.5)	30 (38.4)	0	48 (61.5)	3 (100)	0	0	2 (33.3)	0	4 (66.6)
Nitrofurantoin	3 (3.0)	0	96 (96.9)	9 (11.5)	2	67 (85.8)	0	0	3 (100)	2 (33.3)	1 (16.6)	3 (50.0)
Gentamicin	6 (6.0)	0	93 (93.9)	7 (8.9)	1 (1.3)	70 (89.7)	3 (100)	0	0	2 (33.3)	0	4 (66.6)

Data presented were number (%). Res. - Resistance, Int. - Intermediate, Sen. - Sensitive

Table 4. Association in between different variables with urinary tract infection among diabetic and non-diabetic group

Variables	Urinary tract infection		OR (95%CI)	P-value
	Diabetic	Non-diabetic		
Age				
≥51 years	69	35	3.09 (1.76 to 5.43)	0.0001
≤50 years	42	66		
Gender				
Female	84	86	0.54 (0.26 to 1.09)	0.0866
Male	27	15		
Urine culture				
Positive	111	101	3.71 (2.73 to 5.06)	<0.0001
Negative	219	741		
Organism				
<i>E. coli</i>	99	78	2.43 (1.13 to 5.19)	0.0216
Others	12	23		
Antibiotics *				
β-lactams				
Resistant	186	144	1.42 (1.04 to 1.95)	0.0264
Sensitive	144	159		
Sulphanamides				
Resistant	42	44	0.80 (0.46 to 1.40)	0.4548
Sensitive	66	56		
Quinolones				
Resistant	102	69	1.62 (1.09 to 2.40)	0.0153
Sensitive	120	132		
Nitrofurans				
Resistant	6	17	0.27 (0.10 to 0.73)	0.0102
Sensitive	99	78		
Aminoglycosides				
Resistant	9	10	0.80 (0.31 to 2.08)	0.6604
Sensitive	99	89		
Glycopeptides				
Resistant	3	0	7.00 (0.2548 to 192.27)	0.2496
Sensitive	3	3		

*Data presented were the frequency of sensitivity, which may not be equal to number of sample.

* β-lactams - Amoxicillin, Cefotaxime, Cefixime, Cephalexin, Ceftazidime, Sulphanamides-Cotrimoxazole, Quinolones- Ciprofloxacin, Ofloxacin, Nitrofurans- Nitrofurantoin, Aminoglycosides- Gentamicin, Glycopeptides- Vancomycin

Table 5. Distribution of MDR strains in Diabetic and Non- diabetic patients

Organisms	Diabetic patients		Non- diabetic patients	
	Isolate	MDR n (%)	Isolate	MDR n (%)
<i>E. coli</i>	99	60(60.6)	78	45(57.6)
<i>P. mirabilis</i>	0	0	2	1(50.0)
<i>K. oxytoca</i>	0	0	1	1(100)
<i>K. pneumoniae</i>	3	3(100)	6	4(66.6)
<i>M. morgani</i>	0	0	2	1(50.0)
<i>Acinetobacter spp.</i>	0	0	1	1(100)
<i>Providencia spp.</i>	3	0	3	2(66.6)
<i>Citrobacter spp.</i>	0	0	2	1(50.0)
<i>S. aureus</i>	6	6(100)	4	3(75.0)
<i>Enterobacter spp.</i>	0	0	1	1(100)
<i>P. aeruginosa</i>	0	0	1	1(100)
Total	111	69	101	61

DISCUSSION

Antibiotics are the cornerstones for treating bacterial infections, emergence in resistance of bacteria against antibiotics are main barrier against infection. In this study we have found the pattern of bacteria causing UTI and their antibiotic susceptibility profile in patients with and without diabetes. The patterns of bacteria causing UTI in diabetic patients are similar as in non- diabetic patients and the predominant of the pathogens isolated were Gram negative enteric organisms that commonly cause UTI. The overall prevalence of UTI in patients with and without diabetes was 33.6% and 12.0% respectively. A similar study carried out by Zamanzad *et al.*, showed 20% and 4% of bacterial isolates in diabetic and non-diabetic patients respectively, which is lower than our findings in figure but proportion seems similar (Zamanzad and Moezzi, 2006).

There was higher chance of urine culture positivity in patients with diabetes than non-diabetic patients ($p < 0.05$). This finding suggests that the diabetic patients are at greater risk of UTI than non-diabetic patients. Higher prevalence of *E. coli* both in diabetic and non-diabetic patients seen in this study was comparable with various other similar studies (Chhetri *et al.*, 2001; Akbar, 2001 and Acharya *et al.*, 2015). Another study conducted in India, also found that *E. coli* was the most commonly involved organism (64.3%), followed by *S. aureus* (21.4%) and *K. pneumoniae* (14.3%), which is in almost same in our study as well (Goswami *et al.*, 2001). In this study, majority of the isolates were Gram negative compared to Gram positive isolates. This is due to the predominance of Enterobacteriaceae group. The reason is due to *E. coli* and other enterobacteria are the principal commensals of human gastrointestinal tract and perineum region, and also they are the opportunistic pathogens (Pokharel, 2004) *S. aureus* was only the Gram positive isolates in both diabetic and non-diabetic

patients in this study; *Staphylococcus* isolates was considerably low 5.4% and 4 % in diabetic and non-diabetic patients respectively. This finding is accord with Sibi et al and Mehvish et al. (2011). diabetic patients with UTI; whereas among non-diabetic patients with UTI, gentamicin was the drug of choice with susceptibility of 87.6 % followed nitrofurantoin with susceptibility of 80.4%. Therefore, nitrofurantoin can be recommended as the drug of choice in the empirical initial treatment of UTI in patients with diabetes. Nitrofurantoin was found to be the most effective drug against urinary pathogens also in other similar studies by Maharjan *et al.*, and Gautam et al (2015 and 2002). Resistance of nitrofurantoin against UTI causing bacteria in non- diabetic patients was higher than diabetic patients with UTI ($p < 0.05$). *E. coli* was found to be highly susceptible to nitrofurantoin (96.9% and 85.8 %) and gentamicin (93.9% and 89.7 %) in diabetic and non-diabetic patients respectively in our study. High percentage of *E. coli* isolates was resistant to amoxicillin and this was might be due to the production of β - lactamase by *E. coli* isolates.

Though the lower number of culture positive patients with gram positive bacteria, the effective drug against *S. aureus* was found to be vancomycin (susceptibility of 50%) and gentamicin (susceptibility of 50%) in diabetic patients whereas in non-diabetic patients, the most effective drugs found to be gentamicin (susceptibility of 100%) followed by vancomycin, ciprofloxacin and ofloxacin (75.0 % each). *S. aureus* was highly resistant to amoxicillin, cephalexin, cotrimoxazole, ciprofloxacin and ofloxacin (100% each) followed by ceftazidime (50.0%) in diabetic patients. In non-diabetic group also, it was highly resistant to amoxicillin (75.0%) followed by cotrimoxazole and ceftazidime (50.0 %). The association is also significant in between resistance of β -lactams and UTI ($p < 0.05$). These findings are clearly alarming as our country could be running out of effective antibiotics if this trend continues. In our study, nitrofurantoin had susceptibility of 94.3% followed by gentamicin 91.4% in In our study, UTI was more common among the diabetic patients with the age over 50 (62.2%) and among the non-diabetic patients with age less than 50 (65.3%). This finding correlates the similar result obtained by some other studies (Maharjan *et al.*, 2015; Puri *et al.*, 2006 and Jha and Bapat, 2005). Our finding also showed sexually active age groups might have higher chances of having UTI among non-diabetic patients.

The higher cases of UTI were found in female patients compared to male patients both in diabetic and non- diabetic patients. The majority of the study done all over the world has concluded female predominance to UTI over male (Akbar, 2001; Bonadio *et al.*, 2006; Boroumand *et al.*, 2006 and Geerlings, 2008). The higher rate of occurrence UTIs among female patients is due to the short urethra and its proximity to the anal orifice (Chhetri *et al.*, 2001). However, there was no significant association in between gender and occurrence of UTI among diabetic and non-diabetic patients ($p = 0.086$). According to report from Gondar in 2002, the prevalence of MDR *E. coli* was 65.4% which is similar to our finding (Eshetie *et al.*, 2016). In diabetic patients, 60.6 % ($n = 60$) of *E. coli* isolates were MDR strains and all isolates of *K. pneumoniae* and *S. aureus* were found to be MDR strain; whereas in non-diabetic patients 57.6% ($n = 45$) *E. coli* isolates were found to be MDR strain and 66.6% ($n = 4$) *K. pneumoniae* and 75% ($n = 3$) *S. aureus* were found as MDR strains. Other isolated organisms in non- diabetic patients also found to be

MDR strains but the isolation rate was low during this study period. The rapid development of resistance of antibiotics among the bacteria is attributed to the irrational use of antibiotics and practices of self-medication among general population. Thus, results in the emergence of MDR strains and such drug resistance problem is more prevalent in developing countries due to lack of awareness and lack of effective implementation of the policy that regulates the use of antibiotics. Also due to development of antibiotic resistance against commonly used antibiotics among the bacteria the therapeutic options have become limited and it is a serious issue now. Nevertheless, this study had some limitations; firstly this was hospital based cross-sectional study so only limited sample during limited time interval was studied. Thus the findings may not be generalized to other settings with other normal and diabetic population. Secondly, because of limited resources in this study, only limited type of antibiotic disc was used. Despite these limitations, major strength of our study was we found the recent pattern of bacteria causing UTI and their antibiotic susceptibility profile within this certain locality.

Conclusion

Diabetic patients are relatively at high risk of UTI compare to the non-diabetic patients. *E. coli* was the most predominant organism causing UTI and nitrofurantoin and gentamicin can be the drug of choice in both diabetic and non-diabetic patients. However, periodic monitoring of antibiotic susceptibility pattern of UTI causing pathogen is essential in order to establish reliable up-to date information for rational use treatment of UTI for diabetic and non-diabetic patients.

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