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Uropathogenic Bacteria and Antimicrobial Sensitivity Pattern among Diabetic Patients with Urinary Tract Infection

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Authors' contributions

This work was carried out in collaboration among all authors. All authors contributed in designing the study, analysis of data, interpretation of the results, making the discussion and writing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Urinary tract infection means the presence and active multiplication of microorganisms within the urinary tract that affects any part of urinary tract. A cross sectional descriptive study was conducted on 601 urine sample to determine the antibiotic sensitivity pattern of bacteria causing urinary tract infection in 250 diabetic and 351non-diabetic patients from February 2016 to March 2016. All samples were investigated by standard laboratory procedures. Out of diabetic patient 111 (44.4%) were female and 139 (55.6%) were male and among non-diabetic, 234 (66.7%) were female and 117(33.3%) were male. The UTI prevalence rate was found to be 13% was statistically significant ($p_=$ 0.02), among the significant growth 6.8% diabetic and 6.2% non-diabetic. *Escherichia coli* (54) was the most predominant organism (42.5% in diabetic and 57.5% non-diabetic) followed by *Staphylococcus aureus* (8). Amikacin, Cotrimoxazole and Nitrofurantoin were most sensitive to *E. coli* isolated in diabetic and non-diabetic patients among the tested antimicrobials. High rate of resistance was observed with Norfloxacin and Nalidixic acid. Gentamicin, Cefotaxime,

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Cotrimoxazole and Ciprofloxacin were highly sensitive to *S. aureus* in diabetic patients while Oxacillin and Azithromycin were resistance and in non-diabetic patient highly sensitive antimicrobials were Azithromycin, Gentamicin, Cefotaxime, Cotrimoxazole, Vancomycin and Ofloxacin while Oxacillin was resistance. The antimicrobial sensitivity testing of uropathogenic bacterial isolates should be performed before the initiation of treatment for UTI. Prevalance of uropathogenic bacteria and resistance rate should be monitored regularly.

Keywords: Diabetic; urinary tract infection; Escherichia coli; antimicrobial sensitivity.

ABBREVIATIONS

UTI : Urinary tract infections
MSU : Mid-Stream Urine
DM : Diabetes Mellitus
NDM : Non-Diabetic Mellitus
CLSI : Clinical & Laboratory Standards Institute
S : Sensitive
I : Intermediate
R : Resistant

1. INTRODUCTION

The urinary tract infection (UTI) is one of the most common microbial disease encountered in medical practice affecting people of all age [1]. UTI has been classified by site of infection as upper urinary tract infection and lower urinary tract infection and by severity as complicated and uncomplicated UTIs. Worldwide prevalence of UTI was estimated to be around 150 million persons per year [2]. In Nepal about 20% female experience a single episode of UTI during their lifetime and 3% women have more than one episode of UTI per year [3]. Diabetic patients have a higher incidence of UTI than their non-diabetic counterparts [4,5]. With higher severity UTI, which can be a cause of complications, ranging from dysuria (pain or burning sensation during urination) to organ damage and sometimes even death due to complicated UTI (pyelonephritis) [6]. Potential explanation of the increased UTI in diabetic patients might be the nerve damage caused by high blood glucose levels, affecting the ability of the bladder to sense the presence of urine and thus allowing urine to stay for a long time in the bladder and increasing infection probability [7,8].The major causative organisms are bacteria which are responsible for more than 95% of UTI cases [9]. The most prevalent causative organism of UTI is *Escherichia coli* and is solely responsible for more than 80% of these infections [10]. *Klebsiella, Staphylococci, Enterobacter, Proteus, Pseudomonas,* and *Enterococci* spp. are more often isolated from urine culture. Anaerobic organisms are rarely pathogens in the urinary tract [11]. Coagulase Negative Staphylococci are a common cause of urinary tract infection in some reports [12]. *Staphylococcus saprophyticus* tends to cause infection in young women [13]. Treatment of UTI is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens [14]. The prevalence of antimicrobial resistance among urinary pathogens has been increasing worldwide due to aberrant use of antibiotics in practice [9]. Distribution of urinary pathogens and their susceptibility to antibiotics varies regionally so it becomes necessary to have knowledge of distribution of these pathogens and their susceptibility to antibiotics in a particular setting [15,16]. Incorrect diagnosis, improper use of antibiotics by patients, unnecessary prescriptions, and the use of antibiotics as livestock food additives for growth promotion are the factors contributing towards resistance [17]. Successful antimicrobial therapy of an infection depends on concentration of antibiotic at the site of infection that is high enough to kill or inhibit the growth of microorganism. The choice of drug depends solely on the identification of the species by determination of the sensitivity characteristics of the microorganism. Hence, this study was undertaken to determine the incidence of spectrum of uro-pathogenic bacteria and antimicrobial sensitivity pattern among diabetic and non-diabetic patients with Urinary tract infection.

2. MATERIALS AND METHODS

A total of 601 Clean Catch Mid-Stream Urine (MSU) sample was collected in a sterile urine culture container from diabetic (250) & non diabetic persons (351) from Western Regional Hospital Pokhara, Nepal.

The cross sectional descriptive study was carried out at Microbiology Laboratory of School of Health and Allied Sciences, Pokhara University, Pokhara, Nepal from February 2016 to March 2016. The samples from the patients

were excluded for study who were under antimicrobials medication and for those in which the consent was not obtained. Urine samples were aseptically inoculated by using an inoculating loop of standard dimension obtaining known volume of 0.001 ml of urine for inoculation onto Blood Agar and MacConkey Agar plate and incubated for 24 hours at 37 ± 1^0C. Colony count of more than 10^5CFU/ml were considered significant and further processed for identification. Gram negative bacteria isolated from urine in this study were identified using conventional biochemical tests and antimicrobial susceptibility testing of significant isolates was done by Kirby Bauer disk diffusion method [3]. Carpet culture was performed in Muller Hinton Agar on UTI isolates for Antibiotic Sensitivity test by Kirby Bauer disk diffusion method. Antibiotics used for antibiotic sensitivity pattern were Amikacin (30mcg), Cefotaxime (30mcg), Ciprofloxacin (5mcg), Co-trimoxazole (25mcg), Azithromycin (15mcg), Nitrofurantoin (300mcg), Nalidixicacid (30mcg), Norfloxacin (10mcg), Gentamicin (30mcg), Oxacillin (1mcg), Ofloxacin (5mcg), Novobiocin (30mcg) and Vancomycin (30mcg)(Hi Media, India). After 24 hours incubation at 37 ± 1^0C the antibiotics of the disk diffuses on the agar plate. Each plate was read for zone of inhibition and results were interpreted by following Clinical & Laboratory Standards Institute (CLSI) guidelines [18]. All the data entry, management and statistical analysis was done by using Microsoft Office Excel 2013 and SPSS Version 20.0. P-values of <0.05 were considered statistically significant.

3. RESULTS

Out of 601 patients, the total numbers of female were 345 and male were 256.The total number of diabetic were 250 (41.6%) and 351(58.4%) were non diabetic. Out of 250 diabetic, 111 were female (44.4%) and 139 were male (55.6%). Likewise from 351 non diabetic patients, 234(66.7%) were female and 117(33.3%) were male.

Among the total number of significant growth 78 (13%), 41 (6.8%) shows significant growth in diabetic was found higher than that of non-diabetic 37(6.2%).

Significant growth was found higher above 45 years of age in case of diabetic and 25 to 45 years of age in case of non-diabetic. There is lesser significant growth in diabetic among category of less than 25 years than that of non-diabetic.

From the significant growth, the prevalence of *E. coli* was higher in both diabetic (56.09%) and non-diabetic (83.78%) patients. Overall prevalence of *E. coli, S. aureus, S. saprophyticus, Proteus* spp. were found higher in diabetic than non-diabetic. But the prevalence of *Klebsiella* spp. *Enterobacter* spp. were higher in non-diabetic than diabetic patients.

The total number of gram negative isolates in diabetics were 28 and non-diabetics were 35. Most sensitive drugs in diabetics were Amikacin (60.7%), Nitrofurantoin (53.5%), Cotrimoxazole (53.5%) and Gentamicin (50%). Likewise in non-diabetics most sensitive were Amikacin (68.5%), Cotrimoxazole (62.8%), Nitrofurantoin (54.2%) and Gentamicin (48.37%). Similarly most resistant drugs in diabetics were Nalidixic acid (78.57%), Norfloxacin (64.28%), Ciprofloxacin (60.7%) and Cotrimoxazole (42.85%). In the same way in non-diabetics, resistant drugs were Nalidixic acid (68.57%), Ciprofloxacin (57.1%), Norfloxacin (54.28%) and Cotrimoxazole (37.14%).

In diabetics *E. coli* isolates were most sensitive to Amikacin (60.9%), Nitrofurantoin (56.5%), Cotrimoxazole (52.2%) and resistant to Nalidixic acid (87%), Norfoxacin (69.6%), Ciprofloxacin (69.6%). In non-diabetics all *E.coli* isolates were most sensitive to Cotrimoxazole (61.3%), Amikacin (60.9%), Nitrofurantoin (54.8%) and resistant to Nalidixic acid (71%), Norfloxacin (58.1%), and Cotrimoxazole (38%).

In diabetics *Proteus* spp. isolates were most sensitive to Gentamicin (75%), Ciprofloxacin (75%) and resistant to Norfloxacin (50%), Nalidixic acid (50%). In non-diabetics all *Proteus* isolates were 100% sensitive to all antibiotics.

In diabetic individuals, all *Klebsiella* isolates were sensitive to almost all antibiotics and did not show any resistant pattern. In non-diabetic all isolates were most sensitive to Cotrimoxazole (50%), Amikacin (50%), Nitrofurantoin (50%) and resistant to Nalidixic acid (100%).

The total number of gram positive isolates in diabetic were 13 and non-diabetic were 2. Most sensitive drugs in diabetics were Gentamicin (76.92%), Vancomycin (76.92%), Amikacin (69.23%) and Cotrimoxazole (53.82%). Likewise in non-diabetics most sensitive drug were Azithromycin (100%) and Ciprofloxacin (100%).

Similarly most resistant drugs in diabetic were Amikacin (69.23%), Oxacillin (69.23%) and Azithromycin (69.23%). In the same way in non-diabetics resistant drugs were Cotrimoxazole (7.69%), and Oxacillin (7.69%).

In diabetic, *Staphylococcus aureus*isolates were most sensitive to Gentamicin (85.7%), Cefotaxime (71.4%), Cotrimoxazole (71.4%) and Ciprofloxacin (71.4%) and resistant to Amikacin (57.1%), Oxacillin (57.1%), and Azithromycin (57.1%). Likewise in non-diabetic all isolates were 100% sensitive to Azithromycin, Gentamicin, Cefotaxime, Cotrimoxazole, Vancomycin and Ofloxacin and 100% resistant to Oxacillin.

In diabetics *Staphylococcus saprophyticus* isolates were most sensitive to Vancomycin (100%), Amikacin (100%), Ofloxacin (83.3%), Ciprofloxacin (83.3%) and resistant to Oxacillin (83.5%), Azithromycin (83.5%). Likewise in non-diabetics all isolates were most sensitive to Ciprofloxacin (100%), Azithromycin (100%) and 100% resistant to Cotrimoxazole, Oxacillin, Vancomycin, Cefotaxime, Amikacin and Ofloxacin

4. DISCUSSION

In this study, overall prevalence rate was found 78(13%) out of total cases and was statistically significant ($p_=$ 0.02), among them 6.8% diabetic and 6.2% non-diabetic. In this study significant growth in diabetic cases were higher as compared to non-diabetic cases. This is in accordance with the study done in the Dhulikhel hospital Kathmandu Nepal [19]. Similar type of study was also done in hospital of Bangladesh [20] where sample population was slightly lower than our study. Diabetic patients are more prone to urinary tract infection due to immune compromise, hyper glycosuria and neutrophil dysfunction. However, a study on a large series

Table 1. Sex wise distribution of diabetic and non-diabetic patients

	Gender		Total
	Female	Male	
Diabetic	111	139	250
Non diabetic	234	117	351
Total	345	256	601

Table 2. Significant bacterial growth in comparison with diabetic and non-diabetic patients

	Insignificant Growth	Significant Growth	Total
Diabetic	209	41	250
Non Diabetic	314	37	351
Total	523	78	601

Table 3. Age wise distribution of significant growth

Age Group (years)	Diabetic	Non Diabetic
<25	3	8
25-45	11	18
>45	27	11
Total	41	37

Table 4. Significant uropathogens

Bacteria	Diabetic	Non diabetic
E. coli	23(56.09%)	31(83.78%)
S. aureus	7(17.70%)	1(2.70%)
S. saprophyticus	6(14.63%)	1(2.70%)
Proteus spp.	4(9.75%)	1(2.70%)
Klebsiella spp.	1(2.43%)	2(5.43%)
Enterobacter spp.	0.00%	1(2.70%)
Total	100%	100%

Table 5. Isolated gram negative uro-pathogens with different antibiotics

Organisms N=63	Patient type	*E. coli*			*Proteus* spp.			*Klebsiella*spp.			*Enterobacter*s pp.		
Antibiotics		**N=54 DM =23 NDM =31**			**N=5 DM=4 NDM=1**			**N=3 DM =1 NDM =2**			***N=1* DM=O NDM=1**		
		S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Amikacin (30 mcg)	Diabetic	14	5	4	2	0	2	1	0	0	0	0	0
	Non-diabetic	21	1	9	1	0	0	1	0	1	1	0	0
Ciprofloxacin (5 mcg)	Diabetic	7	0	16	3	0	1	1	0	0	0	0	0
	Non-diabetic	8	5	18	1	0	0	0	0	2	1	0	0
Gentamicin (30 mcg)	Diabetic	11	3	9	3	0	1	0	1	0	0	0	0
	Non-diabetic	16	9	12	1	0	0	0	1	1	0	1	0
Norfloxacin (10 mcg)	Diabetic	5	2	16	2	0	2	1	0	0	0	0	0
	Non-diabetic	11	2	18	1	0	0	0	1	1	1	0	0
Nitrofurantoin (300 mcg)	Diabetic	13	7	3	2	1	1	0	1	0	0	0	0
	Non-diabetic	17	6	8	1	0	0	1	0	1	0	1	0
Nalidixic acid (30mcg)	Diabetic	3	0	20	2	0	2	1	0	0	0	0	0
	Non-diabetic	7	2	22	1	0	0	0	0	2	1	0	0
Cotrimoxazole (25 mcg)	Diabetic	12	1	10	2	0	2	1	0	0	0	0	0
	Non-diabetic	19	0	12	1	0	0	1	0	1	1	0	0

Note: S-Sensitive, I-Intermediate, R-Resistant, DM = Diabetic Mellitus, NDM= Non-Diabetic Mellitus

Table 6. Isolated gram positive uro-pathogens with different antibiotics

Organisms N=15	Patient type	*S. aureus* N=8,Diabetic=7, Non-Diabetic=1			*S. saprophyticus N=7,* Diabetic =6, Non-Diabetic =1		
Antibiotics		**S**	**I**	**R**	**S**	**I**	**R**
Gentamicin (30mcg)	Diabetic	6	1	0	4	1	1
	Non-diabetic	1	0	0	0	1	0
Amikacin (30mcg)	Diabetic	3	0	4	6	0	0
	Non-diabetic	1	0	0	0	1	0
Azithromycin (15mcg)	Diabetic	2	1	4	1	0	5
	Non-diabetic	1	0	0	1	0	0
Cefotaxime (30mcg)	Diabetic	5	0	2	4	0	2
	Non-diabetic	1	0	0	0	0	1
Cotrimoxazole (25mcg)	Diabetic	5	1	1	2	1	3
	Non-diabetic	1	0	0	0	0	1
Vancomycin (30mcg)	Diabetic	4	0	3	6	0	0
	Non-diabetic	1	0	0	0	0	1
Ofloxacin (5mcg)	Diabetic	4	1	2	5	1	0
	Non-diabetic	1	0	0	0	0	1
Oxacillin (1mcg)	Diabetic	3	0	4	1	0	5
	Non-diabetic	0	0	1	1	0	0
Ciprofloxacin (5mcg)	Diabetic	5	0	2	5	1	0
	Non-diabetic	1	0	0	1	0	0

Note: S-Sensitive, I- Intermediate, R-Resistant

of diabetic and non-diabetic patients from a hospital in Italy, the culture positivity rate was 15% and 14% in diabetic and non-diabetic population respectively [21], which is almost similar with our finding (16.4% diabetic and 10.5% non-diabetics). A similar study [7] reported 20% UTI in diabetic patients in their study which is slightly higher than our finding (16.4%). This might be due to the differences in the sample size in these different studies.

It has shown in several studies that women are at increased risk to develop UTI then men [22]. In total sample, majority of the culture positive

patients in our study were also female (57.4%) but in case of diabetic patient majority of culture positive patient were male(55.6%) it might be due to the high number male patient and female might be in antibiotic therapy.

The predominant numbers of pathogens isolated in our study were gram negative bacilli rather than gram positive pathogens. The rate of *E. coli* isolation we found in both diabetic and non-diabetic patients are almost similar in which predominant organism constituted 56% and 83% among diabetic and non-diabetic patients respectively. This is similar with the data obtained by various studies indicated that gram negative bacteria mostly *E. coli* and *Klebsiella*spp. are the predominant pathogens isolated in patients with UTI irrespective of risk factors associated with it [23-26]. This was followed by *Klebsiella*spp. (Diabetic 2.43%; Non diabetic 5.43%) and *Enterococcus* spp. (Diabetic 0%; Non diabetic 2.70%). In another study from Nepal, it was found that *E. coli* was most commonly grown organism (68.7%) followed by *Enterococcus* spp. (13.92%) [27].

The study from India has revealed *Staphylococcus* spp. as the second predominant isolates which is in accordance to our study [28]. There was no difference between the rate of isolation of organisms in diabetic and non-diabetic patients in our study which is in accordance with the study done in Bangladesh [20]. *Pseudomonas* spp. is another gram negative bacterium that is associated with UTI [21]. Irrespective of the status of diabetes and non-diabetic *Pseudomonas* spp. were not isolated from UTI patients in our study.

Regarding the antimicrobial sensitivity profile of the uropathogenes, in our study 69% of the isolated *E. coli* strains were sensitive at similar rate to Amikacin, Gentamicin, Nitrofurantoin, Cotrimoxazole in both diabetic and non-diabetic patient. The significant differences between diabetic and non-diabetic patients to the sensitivity to Gentamycin, Ciprofloxacin and Nitrofurantoin was noted in a study from Bangladesh [20]. But sensitivity to Norfloxacin and Nalidixic acid were slightly different from diabetic and non-diabetic patients. One study was done in Iraq [19] by Abdul Sahib and found Ciprofloxacin resistant *E. coli* significantly higher in diabetic patient but in our study ciprofloxacin resistant *E. coli* significantly higher in non-diabetic patient than diabetic patient. Resistant pattern of *E. coli* in Nalidixic acid was almost similar in both diabetic and non-diabetic patient. This drug is more resistant in most of culture growth. Moreover this difference in sensitivity pattern of isolates could be attributed to time difference between the two studies or environment factors such as practices of self-medications, the drug abuse and indiscriminate misuse of antibiotics among the general population which has favored the emergence of resistance strains.

The limitations of our study were, first information regarding type and duration of diabetes was lacking and second was we could not elaborate the correlation of all the uropathogens among various regions, socioeconomic status, other health status due to the resource management and time factor during the research.

5. CONCLUSION

From the total isolates in this study, the highest prevalence, was of *E. coli* 69.23% (54)and lowest prevalence 1.28% (1 was *Enterobacter* spp. Amikacin, Cotrimoxazole and Nitrofurantoin were highly sensitive to Gram Negative bacteria and resistant to Nalidixic acid and Norfloxacin in both diabetics and non-diabetics. Whereas Gentamicin, Cefotaxime, Cotrimoxazole were most sensitive and Oxacillin, Azithromycin were resistant to Gram positive isolates. *E. coli* is the predominant cause of UTI in both diabetic and non-diabetic patients. Antibiotics that are commonly used for the management of UTI cases are being less effective, so antibiotics should be prescribed only after performing the antimicrobial susceptibility testing.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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