Original Article

Prevalence of Uropathogens in Diabetic Patients and Their Susceptibility Pattern at a Tertiary Care Center in Nepal-A Retrospective Study

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Background: **The incidence of diabetes mellitus round the globe is escalating remarkably. Diabetes mellitus has some deleterious effects on the genitourinary system and render diabetic patients more vulnerable to UTI.** Objective: **This study was undertaken to determine the prevalence of UTI among diabetic patients, the causative pathogens & their antimicrobial susceptibility pattern.** Materials and Methods: **Urine culture & sensitivity test reports data of 462 diabetic patients who had visited the B.P.Koirala institute of health sciences during a five year period, from February 2008 to January 2012, were retrospectively reviewed from log & register book and also from laboratory software(Q-Lab). These data were analyzed statistically using Microsoft Excel 2007.** Results: **The overall prevalence of UTI was 54.76% with female preponderance of 67.98 % and in case of male it was 32.02%. The UTI was common in age group between 31-40 yrs. The most predominant bacterial isolates were *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis and Enterococcus*. In-vitro antimicrobial susceptibility tests revealed that Amikacin was more effective against Gram negative pathogens where as Nitrofurantion was sensitive to Gram positive pathogens. Ampicillin, nalidixic acid and ciprofloxacin were poorly effective against isolated uropathogens.** Conclusions: **This study highlights that the prevalence of UTI among diabetics is considerably high and the responsible pathogens have developed resistance to several potent antibiotics which if not looked into timely, there is every chance that we will be left with no choice in near future. Therefore, it is highly recommended that periodic screening of diabetic patients should be done for optimal therapy and management.**

Key words: UTI, diabetes, bacteria, antimicrobial susceptibility

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by increased blood glucose level resulting from defects in insulin secretion, insulin action, or both[1] .The chronic hyperglycemia in diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Diabetes mellitus has long been considered to be a predisposing factor for urinary tract infection (UTI) and the urinary tract is the principle site of the infection in diabetics with increased risk of complications of UTI.[2, 3]

The incidence of diabetes mellitus is increasing markedly throughout the world and is becoming a serious public health threat particularly in the developing countries. Diabetes mellitus is associated with many complications and in the long run it has some major effects on the genitourinary system which makes diabetic patients more liable to UTI, particularly to upper urinary

tract infections.[4, 5]

Diabetes mellitus has a number of effects on urinary system. Patients either with Type1 DM or Type 2 DM are at increased risk for urinary tract infection. Diabetes causes several abnormalities of the host immune system that may result in a higher risk of infections like UTI.[6]

Even though the precise mechanisms for the predilection of pathogens to cause UTI in diabetics remains unclear, a few research have revealed that the reasons could be immunological impairments such as impaired migration of neutrophils, intracellular killing, phagocytosis, defects in the local urinary cytokine secretions (IL-8, IL-6), increased adherence of the microorganisms to the uroepithelial cells, partly due to a changed and lowered Tamm Horsfall protein, and chemotaxis of polymorphonuclear leukocytes from diabetic patients and neuropathic complications such as impaired bladder emptying, as a result static pools of urine will remain in the bladder. In addition, a higher glucose concentration in the urine acts as a favorable culture medium for pathogenic bacteria and promotes rapid bacterial colonization and growth.[7, 8]

Urine may be static or even bactericidal against uropathogens under certain situations. In diabetes mellitus modification of chemical composition of urine occurs which alters this ability of urine and support the growth of pathogens. It has been experimentally shown that osmotic diuresis secondary to glycosuria predispose to ascending *Escherichia coli* infection in laboratory animals. Autonomic neuropathy in diabetes mellitus impairs bladder emptying and subsequent urological manipulation predispose to UTI. [9]

The successful management of UTI in diabetics 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 and control the spread of infections. Furthermore, the indiscriminate use of antibiotics often results in the increased resistance of urinary pathogens to most commonly used antimicrobials. Updated knowledge of the susceptibility pattern of bacteria is therefore important for the proper selection and use of antimicrobial drugs and for the development of an appropriate prescribing policy.[10]

Owing to the fact that the incidence of UTI is increasing worldwide, this study was undertaken to identify the responsible microbial culprits for the urinary tract infection in diabetic patients including their susceptibility patterns to various antibiotics in a tertiary care teaching hospital of Eastern Nepal that covers the patients of both rural as well as urban areas and provides all emergency management facilities.

Materials and Methods

This is a retrospective study where the urine culture & sensitivity test report data of 462 diabetic patients were collected from laboratory software (Q-LAB) as well as from log & register book for a five year period from February 2008 to January 2012. The data was analyzed statistically using Microsoft Excel 2007 software. The age of all patients included in this survey was above 20 years. Test result of only those patients were included who were clinically diagnosed to be diabetic patients and also had a blood sugar level greater than 7.8 mmol/l and exhibited glycosouria as confirmed by biochemistry laboratory division of our institute.

Clean voided midstream urine samples were collected in sterile containers after giving proper instructions and samples were processed in the laboratory within 2 hours of collection. Urine cultures were performed using semi-quantitative technique whereby urine samples were inoculated on cystein-Lactose electrolyte deficient (CLED) medium plates with a calibrated loop (0.001ml) and incubated at 37°C for 18-24 hours. Urine culture reports that exhibited colony forming units (CFUs) more than 10^5/ml of voided urine were considered positive. For *Staphylococcus aureus*, its pure culture was considered to be significant regardless of the number of CFUs. The pathogens were isolated and identified using phenotypic methods including biochemical testing like catalase, oxidase, coagulase, triple sugar iron agar,sulphide indole motility,citrate,urease etc. Acinetobacter was identified phenotypically based on the motility, fermentative/ oxidative test, utilization of many substrates and other aforementioned biochemical tests. Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion method employing Mueller-Hinton Agar plate as described by the Clinical and Laboratory Standards Institute (CLSI).The antibiotic discs representative of Penicillin group, cephalosporins, aminoglycosides, quinolones and Nitrofurantoin were obtained from Hi-Media Laboratories in the following concentrations: Ampicillin (10μg), Amikacin (30μg), Gentamycin (10μg), Cefotaxime (30μg), ceftriaxone (30μg), Nalidixic acid (30μg) ciprofloxacin (5μg), ofloxacin (5μg), Norfloxacin (10μg) Nitrofurantoin (300μg). These groups of antibiotics have been in use for management of UTI in our hospital. The inoculated plates were incubated at 37°C for 18-24 hours. Diameter of the zone of inhibition around the disc was measured

to the nearest millimeter using a vernier caliper and the isolates were classified as sensitive, and resistant according to CLSI guidelines. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used as reference strains for culture and sensitivity testing.[11, 12]

This study has been approved by the institutional review board of B.P.Koirala Institute of Health sciences, Dharan Nepal.

Results

Out of 462 urine samples report examined in this study, only 253(54.76%) yielded growth while 161 (34.85%) urine sample were sterile, 32(6.93%) grew contaminant and 16(3.46%) had normal skin flora. A major proportion of the samples reported to be sterile which could be due to the fact that these groups of patients are usually placed on antibiotics before sample collection thereby reducing the culture yield of the specimens.[15]And also improper collection of urine specimens give rise to the growth of contaminants and skin flora.

Among those 462 urine samples, 205 (44.37%) were of males and 257 (55.63%) were of females.253 samples demonstrating significant growth of the organisms include 172 female and 81 male. The overall prevalence of urinary tract infection was found to be 54.76% and the prevalence rate was higher in females (67.98%; 172cases) than males (32.02%; 81cases) as depicted in Fig.1
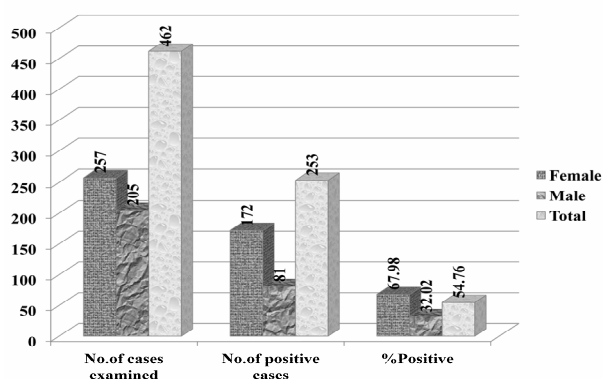

Fig.1   Sex distribution of diabetic patients with UTI

In females, 31-40 age groups were more prevalent to UTI, followed by 41-50 age groups. In males, maximum number of positive cases was observed in 41-50 age groups (Table-1).

Table 1   Prevalence of UTI over the age and sex distribution

Age	Sex		Total No. of cases
	Female	**Male**	
21-30	12	6	18
31-40	105	24	129
41-50	55	51	106
Total	172	81	253

The prevalence of the uropathogens in diabetic patients is shown in Fig 2. The data analysis of reports of the patients showed a considerably high prevalence of *Escherichia coli* infections (49.01%, 124 cases). Among other Gram negative bacilli, 28 (11.07%) were *Klebsiella pneumonia*,21(8.3%) were *Proteus mirabilis*, 15 (5.93%) were *Pseudomonas aeruginosa* and 12(4.74%) were *Acinetobacter calcoaceticus baumanii* complex. whereas among the gram positive cocci *Enterococcus* species (13.84%; 35 cases) predominate followed by *staphylococcus aureus*(7.11%;18 cases).
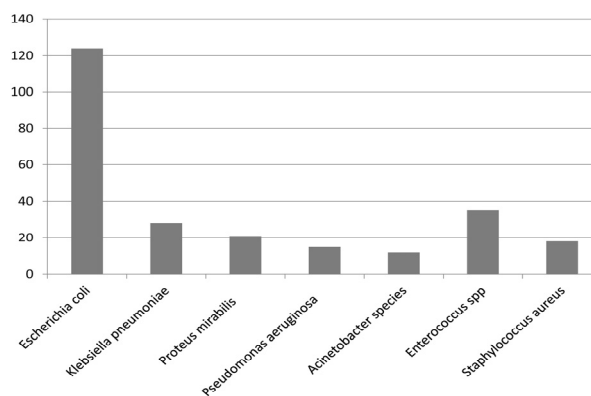

Fig.2   Prevalence of various bacterial pathogens causing UTI in diabetic patients

The antibiotic susceptibility patterns of the isolates to various antimicrobial agents are shown in Table-2.

A total of 10 antibiotics were tested against the isolates and it was observed that amikacin had best inhibitory activity against majority of the isolates except *Acinetobacter* and *Enterococcus*.Gentamicin also demonstrated a considerable degree of sensitivity against the isolates apart from *Enterococcus* and *Proteus*. The gram positive isolates and *Escherichia coli* were found to be sensitive to nitrofurantoin. The Gram positive bacteria; *Staphylococcus aureus* and *Enterococcus spp* were also sensitive to cefotaxime and ceftriaxone. In contrary, a substantial proportion of Gram negative bacteria showed resistance to these cephalosporins. Nalidixic acid was resistant for most of the isolates. Overall, Gram negative

bacilli were found to be more sensitive to both ofloxacin as well as ciprofloxacin than Gram positive cocci. A considerable number of isolates were resistant to both ciprofloxacin & ofloxacin.

Table 2   Antimicrobial susceptibility pattern of the isolated uropathogens from diabetic patients

Organism Isolated	Total No.	S/R	Antimicrobial agents used for Antibacterial Susceptibility Testing									
			AMP	AK	G	CFX	CTR	NA	NOX	OF	CIP	NRF
Potency of Antibiotic discs			10µg	30µg	10µg	30µg	30µg	30µg	10µg	5µg	5µg	300µg
GNB			No.	No.	No.	No.	No.	No.	No.	No.	No.	No.
Escherichia coli	124	S	6	113	95	47	35	23	55	37	26	105
		R	118	11	29	77	89	101	69	87	78	19
Klebsiella pneumonia	28	S	2	23	21	10	8	13	17	15	16	12
		R	26	5	7	18	20	15	11	13	12	16
Proteus mirabilis	21	S	4	16	3	13	12	8	14	17	16	9
		R	17	5	18	8	9	13	7	4	5	12
Pseudomonas aeruginosa	15	S	2	12	11	6	7	2	13	5	13	1
		R	13	3	4	9	8	13	2	10	2	14
Acinetobacter species	12	S	0	2	1	3	2	0	1	2	1	2
		R	12	10	11	9	10	12	11	10	11	10
GPC												
Staphylococcus aureus	18	S	4	16	14	11	13	2	16	13	11	17
		R	14	2	4	7	5	16	2	5	7	1
Enterococcus spp	35	S	7	10	5	17	11	3	8	12	10	28
		R	28	25	30	18	24	32	27	23	25	7
TOTAL	253	S	25	192	150	107	88	51	124	101	93	174
		R	228	61	103	146	165	202	129	152	160	79

AMP= Ampicillin;   Ak= Amikacin;   G=Gentamicin;   CFX=Cefotaxime;   CTR=Ceftriaxone;   Na=Nalidixic acid;   NOX=Norfloxacin;   OF=Ofloxacin;   CIP=Ciprofloxacin and NRF=Nitrofurantoin.

Discussion

In the present study we have attempted to determine the distribution of various bacteria causing UTI among the diabetics, an immunocomprised population, and their antibiotic susceptibility pattern over the last five year period.

In the general population, most urinary tract infections are caused by *Escherichia coli* and affect mainly women because of sexual activity and pregnancy. Prevalence in women is also due to decrease of normal vaginal flora (*Lactobacilli*), less acidic pH of vaginal surface, short & wide urethra, proximity of urethra to anus and poor hygienic conditions.[13]

Nepal is a developing country. Although UTI is one of the common causes of morbidity among Nepalese population, there is a dearth of sufficient national data suggesting the urinary tract infection rate among general population of Nepal to the best of my knowledge. However, in our hospital it has been observed that the prevalence of UTI varies from 25 to 40% in general population.

The prevalence of UTI among the diabetic patients was found to be 54.76%.such higher prevalence were also observed by Saleem M & Daniel B[14], Pargavi B et al[15] and Baloch GH.[16]Contrary to our findings, Geerling et al have reported a prevalence of 26.0% of UTI in dia-betic patients[17] and other studies by Njunda AL et al and patil NR et al have also demonstrated comparatively a lower prevalence of 34.4% & 36% of UTI in diabetics respectively.[18, 19]

The prevalence of UTI among the female diabetic patients was 67.98 %, which was quite higher compared to male (32.02%). This is in agreement with the other reports stating high prevalence of UTI in females.[2, 20,21]

The current study delineated that the occurrence of UTI in diabetics was observed more frequently in subjects between the age of 31 and 40 years followed by the age group 41-50 years. This is in agreement with the study done by Adeyeba et al.[22] Engagement in sexual activity by the adult group and increasing age of the diebetics make them vulnerable to UTI.

The present study revealed that *Escherichia coli* (49%) and *Enterococcus* species (35%) were the most prevalent pathogens followed by *Klebsiella pneumonia*(11%) and *proteus mirabilis* (8%).This finding is similar to other findings which indicate that gram negative bacterium; particularly *Escherichia coli* remains the commonest pathogen isolated in patients with UTI.[2,10,21,14,15,23] In an another study from Nepal, it was found that *Escherichia coli* was the most commonly grown organism (54.5%) , followed by *Staphylococcus aureus* (17.3%), *Enterococcus* species (9.4%) and *Klebsiella* species (7.5%).[24]

Lloyds et al demonstrated that *Enterococcus* spp.

accounted for 35% of urinary tract isolates among gram positive pathogens which is exactly similar to our findings.[25]

The antibiotic susceptibility pattern of the etiological agents in this study revealed that most isolates were resistant to ampicillin, which is relatively cheaper drug and this finding is similar to that of Adeyeba et al.[22] Amikacin has been shown to be the promising antibiotics for UTI in diabetics by Shill MC et al. Nevertheless in our study, except *Acinetobacter* and *Enterococcus* majority of the isolates were found to be sensitive to amikacin. Most isolates were sensitive to gentamicin except *Enterococcus* and *Proteus*. Gram negative pathogens were generally resistant to cephalosporins (cefotaxime and ceftriaxone) while Gram positive cocci were found to be susceptible to these antibiotics which corroborates with the study of shill MC et al.[23] Among quinolones, majority of the pathogens were resistant to nalidixic acid where as a significant number of isolates were resistant to both ciprofloxacin & ofloxacin and approximately equal number of isolates were sensitive to these quinolone duo. In contrast, Nalidixic acid, Ofloxacin and Ciprofloxacin were observed to be more effective against E. coli, K. pneumonia and P. mirabilis respectively in a study.[15] In the present study, Gram negative bacilli were found to be more sensitive to both ofloxacin as well as ciprofloxacin than Gram positive cocci. Dissimilarly in a study, Gram positive cocci were found to be more sensitive to ofloxacin than gram negative bacilli whereas gram negative bacilli were more sensitive than gram positive cocci to ciprofloxacin.[21] Majority of the Gram positive isolates and *Escherichia coli* were sensitive to nitrofurantoin. The most prevalent pathogen, *Escherichia coli* was sensitive to amikacin, gentamicin and nitrofurantoin.

Urinary tract infections are usually treated empirically and culture & susceptibility test are often requested only when the patients fail to improve after the administration of one or more antibiotics. This trend engenders drug resistance in the pathogens. The responsible bacteria especially *P.aeruginosa, Acinetobacter* and *Enterococcus* which are very deft at developing resistance by exploiting various mechanisms can be hard to manage.

Nevertheless, the result of this study may not be representative of the general diabetic population. These data could be used to determine trends in antimicrobial susceptibilities, to formulate local antibiotic policies and to have the alternative choices of antibiotic therapy for the treating physicians to prevent misuse, overuse, and abuse of antibiotics.

Conclusion

This study portrayed that the prevalence of UTI among the diabetic patients was considerably high. Because of the frequency and severity of UTI in diabetics, prompt diagnosis and early treatment is necessary to prevent ensuing complications. It has been deduced that the prevalence of UTI was higher in women with diabetes than in men. Gram negative organisms were most commonly isolated organisms from this group of patients; among which *Escherichia coli* was the principal urinary pathogen. The present study also inferred that antibiotics including ampicillin, nalidixic acid, ciprofloxacin, and ceftriaxone are mostly resistant to the urinary pathogens. The most effective antibiotic was found to be amikacin and nitrofurantoin. In addition, gentamicin, ofloxacin, norfloxacillin and cefotaxime have shown promising level of susceptibility to the urinary isolates from diabetics. The fact that this study has highlighted should incite the policy makers to formulate an antibiotic policy for rational use of antibiotics. However, regular monitoring of susceptibility pattern of urinary pathogens is essential to establish reliable information for optimal empirical therapy of diabetic patients with UTI.

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