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Frequency and risk factors of UTI and susceptibility pattern of uropathogen and impact of glycaemia control among diabetic patients.

Rubina Sabir[1], Anum Butt[2], Kulsoom Baqa[3], Ambreen Shiraz[4], Nasreen Aziz[5]

1. MSc (Biochemistry)
Laboratory In-charge
Department of Clinical and Research Laboratory
Baqai Institute of Diabetology and Endocrinology
Baqai Medical University Karachi, Pakistan.
2. M.Phil
Research Officer
Research Department
Baqai Institute of Diabetology and Endocrinology
Baqai Medical University Karachi, Pakistan.
3. M.Phil
Research Officer
Research Department
Baqai Institute of Diabetology and Endocrinology
Baqai Medical University Karachi, Pakistan.
4. BDS
Lecturer Biochemistry
Baqai Medical University, Karachi.
5. M.Sc
Department of Clinical and Research Laboratory
Baqai Institute of Diabetology and Endocrinology
Baqai Medical University Karachi, Pakistan.

Correspondence Address:
Rubina Sabir
Plot No. 1-2, II-B,
Nazimabad No 2, Karachi, Pakistan
laboratory@bide.edu.pk

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ABSTRACT… Objectives: The present study is designed to determine the frequency and risk factor of urinary tract infection in patients with or without diabetes, and to determine antimicrobial susceptibility among type 2 subjects with controlled and uncontrolled glycemia. **Study Design:** Retrospective Study. **Setting:** Department of Microbiology, Baqai Institute of Diabetology and Endocrinology (BIDE). **Period:** October 2016 to September 2018. **Material & Methods:** Ethical approval was obtained from the institutional review board of BIDE. Total 549 patients were included in the study. **Results:** A total of 117 (28.96%) and 287 (35.52%) uropathogens were isolated from patients with and without diabetes respectively. Percentage of positive culture was high which was 62.30% and 38.25% in female in both patients with and without diabetes respectively. E. coli was found to be the most frequently isolated bacteria from both patients with diabetes and without diabetes, as 50.43% and 52.26% respectively. Since, Patients with diabetes have a higher prevalence of UTI than non-diabetics. **Conclusion:** Hence, preventive measures should be taken and patients should be properly guided about the complications of diabetes and recurrent infections.

Key words: Diabetes Mellitus, E.coli, Urinary Tract Infections, Uropathogens.

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INTRODUCTION

Diabetes mellitus (DM) is the serious and common endocrine disease[1] have several etiologies characterized by increased plasma glucose levels with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[2] The rate of morbidity and mortality and its prevalence has risen dramatically worldwide[3,4,5] especially for lower middle-income countries. In Pakistan its prevalence was predicted to increase 67% from 2010 to 2030.[6] It has severe impact on cardiovascular system, kidneys, nerves and eyes.[4] Additionally, a chronic and complex illness which demands continuous medical as well as self-care.[2]

DM was associated with many complications and immunocompromised situation to fight against pathogens which may lead to increase the risk of certain infections, especially foot infections, yeast infections, surgical site infections and urinary tract infections.[1,3] The urinary tract infection (UTI) covers variety of clinical entities.[3] It is the most common microbial disease affecting people of all ages and its prevalence varies 0.7% to 20% worldwide.[7,8]

UTI is the common infection observed in patients with DM[5] and about 35% patients are reported highly susceptible to it.[7,8] Poor bladder function, obstruction in urinary flow and incomplete voiding are commonly found in patients with diabetes increasing the risk of UTI.[3] The disorder of autonomic nervous system leads to incomplete bladder emptying, which allows uropathogens to colonize and presence of urinary glucose coupled with poor metabolic control, provides environment for pathogens to flourish and cause UTI in patients with diabetes.[7] Microvascular complications like neuropathy and glycosuria can also predispose to infections like UTI.[1] However,

good glycemic control in patients with diabetes may prevent from UTI.[8] Prevalence of UTI was reported around four times higher in patients with diabetes, also females with diabetes have two to three-fold higher risk of UTI as compared to females without diabetes.[3] It is reported that UTI increases fivefold higher mortality risk in elderly patients with diabetes as compare to non-diabetic geriatric patients.[1]

The most common Uropathogens isolated from urine specimen of patients with diabetes are Escherichia coli, Proteus, Klebsiella, Staphylococcus aureus, Streptococcus and Candida albicans.[3,7,8,9]

There was an increase in antimicrobial resistance found among uropathogens both in community and hospital settings causing difficulty in the treatment of UTI.[3,5] Multi drug resistance (MDR) bacteria were E.Coli and Klebsiella Pneumoniae.[3]

The present study is designed to determine the frequency and risk factor of urinary tract infection in patients with or without diabetes and to determine antimicrobial susceptibility among type 2 subjects with controlled and uncontrolled glycemia.

MATERIAL & METHODS

This study was conducted at Microbiology Department, Clinical and Research Laboratory of Baqai Institute of Diabetology and Endocrinology (BIDE), a 24 hours' laboratory service in Karachi-Pakistan between October 2016 to September 2018. Ethical approval was obtained from the institutional review board (IRB) of BIDE. Keeping the ratio of Diabetic to non-diabetic as 1:2[3], total 549 (183 patients with and 366 patients without diabetes) out of 1256 screened patients were included in the study.

Medical record was obtained from the HMS (Hospital management system) of BIDE. Age 20 years and above included in the study.

Patients were divided into two groups and group 2 was further divided in two subgroups;

1. Patients with diabetes
2. Patients without diabetes
   a. Controlled glycemia
   b. Uncontrolled glycemia

Isolation of Uropathogens

Mid-stream clean catch urine specimens[10] were collected from patient and incubated for 24 hours or overnight at temperature of 35°C after inoculation on Cled medium (Oxoid) and Blood agar (Oxoid) plates.[7] Growth of organisms was observed and identification tests were done.[1,2]

Identification of Uropathogens

Gram negative organisms were identified by using Triple Sugar Iron (TSI), citrate utilization, Sulphide Indole Motility media (SIM) and urea hydrolysis.[3,4] Mueller-Hinton agar was used for antibiogram test.[4,5] Susceptibilities of the common isolated bacteria (E. coli, Klebsiella pneumoniae, Proteus species, Staphylococcus aureus, and Proteus mirabilis, Enterobacter species and Pseudomonas aeruginosa) to selected antimicrobial agents causing UTI were examined. Susceptibility testing was done by using the Clinical and Laboratory Standards Institute (CLSI) guidelines recommended modified Kirby-Bauer disc diffusion method with commercial antibiotic discs (Oxoid).

Antimicrobial Susceptibility Testing

The antibiotic discs of clavulanic acid (AMC) 30 µg, piperacillin/tazobactam (TZP) 110 µg, cefotaxime (CTX) 30 µg, cefpirome (CPO) 30 µg, sulbactam-cefoperazone (SCF) 105 µg, vancomycin (VA) 30 µg, imipenem (IPM) 10 µg, amikacin (AK) 30 µg, erythromycin (E) 15 µg, clarithromycin (CLR) 15 µg, clindamycin (DA) 2 µg, ciprofloxacin (CIP) 5 µg, sulphamethethoxazole (SXT) 25 µg, fosfomycin (FOS) 50 µg, fusidic acid (FD) 10 µg and doxycycline (DO) 30 µg, discs were used to assess the antimicrobial susceptibility. The inhibition zones were interpreted according to the CLSI guideline (CLSI, 2011).[2,6]

RESULT

Out of 549 analyzed urine specimens, 68 (12.39%) were sterile cultures, 87 (15.85%) were found with the growth of mix insignificant bacteria and 394

(71.77%) were positive for uropathogens. A total of 117 (28.96%) and 287 (35.52%) uropathogens were isolated from patients with and without diabetes respectively. Percentage of positive culture was high which was 62.30% and 38.25% in female in both patients with and without diabetes respectively (Table-I).

Among patients with diabetes, 1.64%, 4.92%, 24.59%, 46.45%, 20.77% and 1.64% belonged to age group equal or less than 20 years, between 31 - 35 years, 36 - 50 years, 51 - 65 years, 66 - 80 years and above 81 years respectively. It was found that female was more prone to have UTI among these 6 groups of patients with diabetes. (Table-II)

(Table-III)The number of positive cultures was very high in uncontrolled glycemic group which was 145 (79.23%).

E. coli (in Gram negative) and S aur (in Gram positive) were the most predominant uropathogen isolated in both patients with and without diabetes. In the urine sample of patients with diabetes E coli was found 50.43%, K pn 13.68%, Pr mir 6.84% and S aur 5.13% whereas in patients without diabetes E coli 52.26%, K pn 19.86%, and S aur 6.27%. (Table-IV)

E. coli and K pn exhibited maximum sensitivity against Sulbactam-cefoperazone (100%), Fosfomycin (100% and 70% respectively) and Imipenem (97.92% and 100% respectively) followed by Amikacin (87.72% and 81.25% respectively) and Piperacillin / Tazobactam (85.96% and 87.50% respectively) in patients with diabetes. Nitrofurantoin showed 88.89% sensitivity to K pn) in patients with diabetes. Ceftazidime, Piperacillin/Tazobactam, Sulbactam-cefoperazone, Imipenem, Ciprofloxacin and Fosfomycin exhibited over 100% sensitivity for P mir.

Clavulanic acid, Piperacillin / Tazobactam, Sulbactam-cefoperazone, Vancomycin, Amikacin, Clarithromycin and Nitrofurantoin showed 100% sensitivity to S aur (Table-V).

	Positive Culture			
	Patients With Diabetes		Patients Without Diabetes	
	n	%	n	%
Male	35	30.70	45	16.07
Female	79	69.30	235	83.93
Total	114	62.30	280	38.25
	Negative Culture			
	Patients With Diabetes		Patients Without Diabetes	
	n	%	N	%
Male	29	42.03	20	23.26
Female	40	57.97	66	76.74
Total	69	37.70	86	11.75

Table-I. Gender wise distribution of positive and negative urine specimens

	Patients With Diabetes				Patients Without Diabetes			
Age (yrs)	n	%	Positive (n)	Positive (%)	n	%	Positive (n)	Positive (%)
≤ 20	3	1.64	1	0.88	29	3.96	19	3.39
21-35	9	4.92	4	3.54	231	31.56	172	30.71
36-50	45	24.59	25	22.12	60	8.20	51	9.11
51-65	85	46.45	57	50.44	30	4.10	25	4.46
66-80	38	20.77	24	21.24	16	2.19	13	2.32
≥ 81	3	1.64	2	1.77	0	0.00	0	0.00

Table-II. Distribution of patients according to age groups

Glycemic Status	Age (years)	Total		Male		Female	
		n	%	n	%	n	%
Controlled	≤ 20	0	0.00	0	0.00	0	0.00
	21-35	1	2.63	0	0.00	1	100.00
	36-50	11	28.95	2	18.18	9	81.82
	51-65	15	39.47	7	46.67	8	53.33
	66-80	10	26.32	6	60.00	4	40.00
	≥ 81	1	2.63	0	0.00	1	100.00
	Total	38	20.77	15	39.47	23	60.53
Uncontrolled	≤ 20	3	2.07	1	33.33	2	66.67
	21-35	8	5.52	0	0.00	8	100.00
	36-50	34	23.45	8	23.53	26	76.47
	51-65	70	48.28	24	34.29	46	65.71
	66-80	28	19.31	15	53.57	13	46.43
	≥ 81	2	1.38	1	50.00	1	50.00
	Total	145	79.23	49	33.79	96	66.21

Table-III. Age and gender wise distribution of patients with controlled and uncontrolled diabetes

Uropathogens	Patients With Diabetes (n=117)		Patients Without Diabetes (n=287)	
	(n)	%	(n)	%
Gram Negative				
Escherichia coli	59	50.43	150	52.26
Klebsiella pneumoniae	16	13.68	57	19.86
Proteus mirabilis	8	6.84	13	4.53
Enterobacter Species	5	4.27	5	1.74
Pseudomonas aeruginosa	2	1.71	7	2.44
Proteus vulgaris	1	0.85	3	1.05
Proteus species	0	0.00	11	3.83
Pseudomonas species	0	0.00	3	1.05
Morganella morganii	0	0.00	3	1.05
Gram Positive				
Staphylococcus aureus	6	5.13	18	6.27
Enterococcus species	2	1.71	0	0.00
Streptococcus species	0	0.00	2	0.70
Yeast				
Candida species	15	12.82	12	4.18
Candida albicans	3	2.56	3	1.05

Table-IV. Distribution of uropathogens in patients with and without diabetes

		Gram Negative					Gram Positive	
Antimicrobial Agent	Susceptibility pattern	E coli	K pn	Pr mir	Ebc spp	Ps aer	S aur	Enc spp
Ampicillin	S	4.35	50.00	--	100.00	0.00	--	--
	R	95.65	50.00	--	0.00	100.00	--	--
Amoxicillin	S	11.11	7.69	--	66.67	--	--	--
	R	88.89	92.31	--	33.33	--	--	--
Clavulanic acid	S	58.82	53.85	28.57	100.00	0.00	100.00	50.00
	R	41.18	46.15	71.43	0.00	100.00	0.00	50.00
Ceftazidime	S	--	--	100.00	--	0.00	--	--
	R	--	--	0.00	--	100.00	--	--
Piperacillin / Tazobactam	S	85.96	87.50	100.00	80.00	100.00	100.00	50.00
	R	14.04	12.50	0.00	20.00	0.00	0.00	50.00
Cefuroxime	S	44.44	25.00	--	50.00	--	0.00	--
	R	55.56	75.00	--	50.00	--	0.00	--
Cefotaxime	S	39.39	55.56	--	33.33	0.00	0.00	--
	R	60.61	44.44	--	66.67	100.00	0.00	--
Ceftriaxone	S	37.50	80.00	--	--	--	0.00	--
	R	62.50	20.00	--	--	--	0.00	--
Sulbactam-cefoperazone	S	100.00	100.00	100.00	80.00	100.00	100.00	50.00
	R	0.00	0.00	0.00	20.00	0.00	0.00	50.00
Vancomycin	S	--	--	--	--	--	100.00	100.00
	R	--	--	--	--	--	0.00	0.00
Imipenem	S	97.92	100.00	100.00	100.00	50.00	100.00	50.00
	R	2.08	0.00	0.00	0.00	50.00	0.00	50.00
Amikacin	S	87.72	81.25	88.89	20.00	100.00	100.00	0.00
	R	12.28	18.75	11.11	80.00	0.00	0.00	100.00
Erythromycin	S	--	--	--	--	--	33.33	50.00
	R	--	--	--	--	--	66.67	50.00
Clarithromycin	S	--	--	--	--	--	100.00	50.00
	R	--	--	--	--	--	0.00	50.00
Clindamycin	S	--	--	--	--	--	60.00	0.00
	R	--	--	--	--	--	40.00	100.00
Nalidixic acid	S	10.87	30.00	75.00	20.00	0.00	0.00	0.00
	R	89.13	70.00	25.00	80.00	100.00	100.00	0.00
Norfloxacin	S	14.29	40.00	75.00	0.00	100.00	0.00	0.00
	R	85.71	60.00	25.00	100.00	0.00	100.00	0.00
Ciprofloxacin	S	24.56	37.50	100.00	20.00	100.00	60.00	50.00
	R	75.44	62.50	0.00	80.00	0.00	40.00	50.00
Sulphamethoxazole	S	32.65	36.36	75.00	20.00	0.00	40.00	50.00
	R	67.35	63.64	25.00	80.00	100.00	60.00	50.00
Fosfomycin	S	100.00	70.00	100.00	100.00	100.00	50.00	50.00
	R	0.00	30.00	0.00	0.00	0.00	50.00	50.00
Moxifloxacin	S	23.4	50.00	100.00	75.00	100.00	75.00	--
	R	76.60	50.00	0.00	25.00	0.00	25.00	--
Nitrofurantoin	S	61.67	88.89	--	100.00	0.00	100.00	--
	R	8.33	11.11	--	0.00	100.00	0.00	--
Fusidic acid	S	--	--	--	--	--	--	50.00
	R	--	--	--	--	--	--	50.00
Doxycycline	S	--	--	--	--	--	--	100.00
	R	--	--	--	--	--	--	0.00

Table-V. Antimicrobial susceptibility pattern of uropathogens isolated from patients with diabetes

S = Sensitive, R = Resistant, E coli = Escherichia coli, K pn = Klebsiella pneumoniae, Pr mir = Proteus mirabilis, Ebc spp = Enterobacter Species, Ps aer = Pseudomonas aeruginosa, S aur = Staphylococcus aureus and Enc spp = Enterococcus species.

DISCUSSION

This study was conducted among 549 patients (183 patients with diabetes and 366 patients without diabetes). Similar study was done by Viral R Dave et al with 300 patients [200 non-diabetic and 100 diabetics].[3] Al-Asoufi A et al study comprises unequally distributed samples among 116 [37 patients with diabetes and 79 patients without diabetes] patients.[11]

In present study the frequency of positive cultures was as high as 62.30% in patients with diabetes whereas the prevalence of UTI among patient with diabetes was found to be 54.76% in Pramod kumar Jha et al study.[12] The prevalence of UTI among patient with diabetes was found to be 35 % in Mohammed Abdul-Imam Almazini's study[13], 33.8% in Mama M et al study[14], 22.6% in Woldemariam HK et al study[15],

22.0% in Nabaigwa BI et al study[7] and 13.8% in Nigussie D and Amsalu A study on diabetic UTI.[16] The variation of the frequency of positive cultures / UTI may be due to the differences of the environment, social habits of the community and habits of personal hygiene of each study area.
Positive cultures were 62.30% in patients with diabetes and 38.25% in patients without diabetes in our study. The results from Viral R Dave et al study showed cases having positive urinary culture were 92% and 67% in diabetic and nondiabetic groups, respectively.[3] The overall prevalence of UTI in patient with diabetes and without diabetes was 34.5% and 26.7% respectively in Acharya D et al study[17] whereas the incidence of UTIs in patient with diabetes were maximum than nondiabetics in the Nureen Zahra et al study.[10] The reason of this difference in rate of bacterial UTI etiologies is may be due to differences in geography, social habits of the community, personal hygiene and health education practices.

Female were more prone to have UTI.[10] In present study, the percentage of positive culture was 62.30% and 38.25% in female in both patients with and without diabetes which is alarming. Significant bacteriuria in female patients with diabetes was found to be 83.9%, 63.7% and 23% in Mama M et al, Nureen Zahra et al and Abate et al studies respectively.[10,14,18] Decrease of Lactobacilli (the normal vaginal flora), poor hygienic condition, short and wide urethra, proximity to anus and less acidic pH of vaginal surface might be responsible for high infection rate in female population.

Among patients with diabetes 24.59% belonged to group age 36 - 50 years in our study. Eighteen percent of patients were between the age 31 and 45 years in Viral R Dave et al study (3). Among patients with diabetes 46.45% belonged to group age 51 - 65 years in our study. 78% belonged to more than 45 years of age in Viral R Dave et al study.[3]

Among patients with diabetes 20.77% belonged to group age 66 - 80 years in our study and 79.23% positive samples belongs to uncontrolled glycemic group. Similar results found in Nabaigwa BI et al[7] and Sewify M et al studies[8] whereas Mama M et al study showed 97.50% UTI in uncontrolled glycemic group.[14] Only 20% isolates were obtained from participants with a normal glucose level in Nabaigwa BI et al study.[7]

E. coli was found to be the most frequently isolated bacteria from both patients with and without diabetes, as 50.43% and 52.26% respectively, in present study. Similar findings were observed from many other studies.[3,5,10,18,19,20] E coli was also the most common organism isolated in urinary culture of Diabetic 87% and non-diabetic group 54.5% in Viral R Dave et study.[3] In Nureen Zahra et al study E. coli was detected in 60% Diabetic patients and 32%in nondiabetic patients.[10] The most common organism isolated was E. coli in 22.58% Diabetics and in 5.38% among healthy control group in Gurjar D et al study.[5] In the Md. Hamza Saber et al study the rate of E. coli isolation in the diabetic males and females (57.5% and 63.8%) was lower than nondiabetic male and female [83.3% and 76.1%].[19] But the results of

Al-Asoufi A et al study showed 15.5% E coli in diabetic and 29.3% in non-diabetic patients.[11]

Another most prominent uropathogen found in Sewify M et al study[8] also in present study was Klebsiella pneumoniae which was found to be 13.68% and 19.86% in patients with diabetes and without diabetes respectively. Klebsiella was isolated in urinary culture of patient with diabetes 1.1% and non-diabetic group 9.7% in Viral R Dave et study.[3] The contribution of Klebsiella species 25.1% in Murmu M et al study[20] In Sharma S et al study on UTIs in patient with type 2 diabetes aged over 60 years frequency of Klebsiella was16.3% (1). 8.6 % were Klebsiella spp. in Nabaigwa BI et al study among diabetic patients with UTI.[7]

Pseudomonas was isolated in urinary culture of 4.3% diabetic patients and 6.7% of non-diabetic group in in Viral R Dave et study.[3] We found 1.71% and 2.44% of Pseudomonas in patients with and without diabetes respectively. According to Murmu M et al study the contribution of Pseudomonas in UTI of Diabetic patients was 14.2%.[20] In Sharma S et al study on UTIs in patient with diabetes aged over 60 years' frequency of Pseudomonas was found to be 2.3%.[1]

In Gurjar D et al study Enterobacter was 1.08%[5] and in our study it was 4.27% in patients with diabetes.

Frequency of Staphylococcus aureus in diabetic patients was found higher 38.2% than 22.2% in non-diabetics.[19] The contribution of Staphylococcus was 7.5% Murmu M et al study.[20] Our study results showed S aur 5.13% in the urine sample of patients with diabetes and S aur 6.27% in patients without diabetes. In Nureen Zahra et al study S aureus was 2.4% in diabetic patients and 1.2% in nondiabetic patients.[10] The contribution of Staphylococcus 2.15% Gurjar D et al study.[5]

In Sharma S et al study on UTIs in type 2 diabetics aged over 60 years, frequency of Enterococci was 9.3%.[1] Enterococci was isolated in urinary culture of Diabetic 4.3% and non-diabetic group 11.9% in Viral R Dave et study.[3] Only 2.85% and 2.15% enterococci found in Nabaigwa BI et al and Gurjar D et al studies.[5,7] Our study results showed 1.71% in diabetic patients.

In Nureen Zahra et al study Candida was detected in 23.7% Diabetic patients and 37.5% in nondiabetic patients.[10] The contribution of Candida species was 18.4% in Murmu M et al study.[20] We found Candida spp in 12.82% patients with diabetes and 4.18% in patients without diabetes. In Woldemariam HK et al study, the overall prevalence of significant candiduria was 7.7%.[15] Candida was 3.3% isolated in urinary culture of patients with diabetes as and as 6.0% in patients without diabetes Viral R Dave et study.[3] In Sharma S et al study on UTIs in type 2 diabetics aged over 60 years frequency of Candida was 2.3%.[1]

E. coli and K pn exhibited maximum sensitivity against Sulbactam-cefoperazone, Fosfomycin, Imipenem, Amikacin and Piperacillin / Tazobactam in patients with diabetes. Similar results were found in Viral R Dave et, Gurjar D et al and Murmu M et al studies.[3,5,20]

CONCLUSION

Patients with diabetes have a higher prevalence of UTI than without diabetes, also the diabetic females are more susceptible to bactosuria. Hence, preventive measures should be taken and patients should be properly guided about the complications of diabetes and recurrent infections. Patients should be informed how necessary it is to achieve glycemic control and how it can prevent multiple recurrent infections.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Rubina Sabir	Conception design, Literature search, interpretation of data, wrote and approved the manuscript.	
2	Anum Butt	Design, Literature search, Interpretation of data, Wrote the manuscript.	
3	Kulsoom Baqa	Literature search and edited the manuscript.	
4	Ambreen Shiraz	Wrote and edit the manuscript.	
5	Nasreen Aziz	Literature search and wrote the manuscript.	