PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF URINARY TRACT PATHOGENS AMONG DIABETIC MELLITUS DISEASE PATIENTS IN BENIN CITY, EDO STATE

[1*]**Moses-Otutu, I. M. and [1]Emoregan, A. S.**
Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria
*Correspondence to: ifueko.moses-otutu@uniben.edu; +2348036868229

Received: 31st July, 2021 **Accepted:** 17th August, 2021 **Published:** 31st December, 2021

ABSTRACT

Background: Urinary tract infection caused by more resistant pathogens in patients with diabetic mellitus are more severe and is associated with worse outcomes than in patients without diabetes mellitus.

Aim: To determine the prevalence of urinary tract pathogen, its associated risk factors and antimicrobial susceptibility pattern among diabetic mellitus patients in Benin City.

Methodology: Two hundred diabetic mellitus disease patients consisting of 62 males and 138 females within the age range 16 to 99years constituted the study group. Clean catch midstream urine sample was collected and viewed macroscopically, cultured and incubated aerobically at 37^0C for 24hrs. Bauer and Kirby antimicrobial susceptibility test was done on isolated pathogens. The results obtained were analyzed statistically.

Results: A 28.5% UTI was obtained. Gender and Age of participants significantly imparted on the prevalence of UTI ((p=0.001; p=0.009). Educational status, marital status, number of years infected with DM and occupation significantly influenced the acquisition of UTI (p=0.009; p=0.030; p=0.023; p=0.001); Mode of residence did not significantly influence the prevalence of UTI (p = 0.356). The Gram negative organisms were less inhibited by the cephalosporins and fluoroquinolones while the Gram positive organism was susceptible to the cephalosporins (ceftazidime and cefuroxime) and fluoroquinolones (ofloxacin and levofloxacine).

Conclusion: Prevention of infection and early detection of UTI is the key to reducing complications from bacterial infections in Diabetic mellitus disease patients.

Keywords: UTI, Risk factors, Antimicrobial susceptibility pattern, Diabetes mellitus.

INTRODUCTION

Diabetes Mellitus (DM) is defined as a chronic disease which occurs when the pancreas does not produce enough insulin (ADA, 2012). It is also a condition when the body cannot effectively use the insulin it produces leading to an increased concentration of glucose in the blood called hyperglycemia (Sherwin and Jastreboff, 2012). The glucose levels build up in the blood and urine of diabetes mellitus patients results in excessive urination, thirst, hunger and problems with fat and protein metabolism. (Mnif *et al.,* 2013). Moreso, the high glucose levels in diabetics urine improve the growth of bacteria in the urine making diabetics to have higher severity of 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) (Saleem and Daniel, 2011; Johnson *et al.*, 2013). Other conditions such as incomplete bladder emptying due to autonomic neuropathy and high glucose concentration in the urine allow urinary colonization by microorganisms (ChinHong, 2006). Evidences also abound that patients with diabetes have an increased risk of asymptomatic bacteria and urinary tract infections (UTIs) with UTIs being the most common bacterial infections in diabetic patients (Bonadio *et al*., 2006).

A patient is said to have urinary tract infection when there is significant bacteriuria in their urine (Vasudevan, 2014). Significant bacteriuria is defined as a quantitative urine culture yielding greater than or equal to 10^5 Colony Forming Unit (CFU) per milliliter of urine (Oslen *et al.,* 2000). Urinary tract infections (UTI) are common among patients with diabetes mellitus; In these patients, UTI caused by more resistant pathogens are more severe and is associated with worse outcomes than in patients without diabetes mellitus (Mnif *et al.,* 2013). Moreso, Diabetic patients have a higher incidence of UTI, upper UTIs and longer hospitalization than their non diabetic counterparts (Geerlings, 2008 and De Lastours and Foxman, 2014).

A 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 (Geerlings *et al*., 2002; Mnif *et al.,* 2013). Another explanation is that high glucose levels in urine improve the growth of the bacteria in the urine (Johnson *et al*., 2013). An epidemiological study can help in understanding the contribution of urinary tract pathogens, its associated risk factors as well as knowing the antimicrobial susceptibility pattern of UTI isolated among diabetic mellitus patients as an important factor at reducing the burden of the disease among infected patients in our locality; Hence, this study.

MATERIALS AND METHODS

Study Area

This study was conducted at a Secondary health institution located within Benin City, Edo State, Nigeria. The secondary health facility has a referral status from primary health facilities in Edo State.

Study population

Randomly selected known diabetic mellitus disease patients consisting of 62 males and 138 females within the age range 16years to 99years were recruited for this study. Approval for this research was obtained from the Ethics and Research Committee Ministry of Health, Edo State, Nigeria. Informed consent was also obtained from all the study participants before sample collection. Structured questionnaire was used to obtained demographic and other relevant data from each participant.

Sample collection

Each participant was given a wide-mouth sterile universal containers; into which a "clean catch" midstream urine of about 10 – 20 ml was collected (Cheesbrough, 2006).

Isolation and Identification

Each urine sample was aseptically inoculated into Blood agar, Mac Conkey and Sabouraud Dextrose agar and aerobically incubated at 37^0C for 24hours. Emergent colonies were counted, and a single colony represents one colony forming unit (cfu) where 100 colonies or more represents significant bacteriuria count while less than 10^5 were not significant. Morphological identification test, microscopic examination of the Gram stained preparation was also carried out. Proteus sp was used as positive control. For yeast cells, Gram staining and germ tube test was carried out. *Candida albicans* was identified by its ability to produce germ-tubes when incubated in serum at 37^0C for 2hours. Biochemical Identification was further carried out for identification and confirmation of isolates. Kirby-Bauer disc diffusion method was used for antibiotic susceptibility test (Cheesbrough, 2006).

Statistical Analysis

The categorical variables obtained from laboratory analysis were tabulated, encoded and analyzed statistically using Statistical Package for Social Sciences (SPSS version 16) program. Chi square was used as test of significance and levels of significance were accepted at $p<0.05$.

RESULTS

Out of the 200 urine samples examined, 57 (28.5%) were positive for urinary tract infection as they had significant bacteria in their urine while 143 (71.5%) were negative for UTI.

Of the 200 (100%) urine samples examined 62 (31.0%) were males, 138 (69.0%) were females. The study participants within the age group <20years constituted 6 (3.0%) while age groups 21-40years constituted 23 (11.5%), 41-60years age groups were 120 (60.0%) with >60years age group constituting 51 (25.5%). Those having primary education comprised 34 (17.0%), while those with secondary education were 80 (40.0%) and those with tertiary education comprised 86 (43.0%).

The study participants was made of 8 (4.0%) single participants and 192 (96.0%) married participants. Those infected with DM less than five years comprised 84 (42.0%), with those infected within 6-9years constituting 104 (52.0%) while those infected with DM for 9-12years constituting 12 (6.0%). Participants residing in rented apartments comprised 109 (54.5%) while those residing in their own house comprising 91 (45.5%). Participants that were artisans constituted 21 (10.5%), civil servants were 6 (3.0%), those farming were 4 (2.0%), self employed participants comprised 157 (78.5%) while the retiree made up 12 (6.0%).

Only 5 organisms were isolated from the participants that had UTI and they comprised 4 bacteria and 1 Candida species. The isolates comprised *E. coli* 19 (9.5%), *Staphylococcus aureus* 13 (6.5%) with *Proteus vulgaris* comprising 9 (4.5%) and Klebsiella species recording 8 (4.0%) while only 1 *Candida albicans* was isolated 8 (4.0%). Isolates from in-patients constituted *E. coli* and *Staphylococcus aureus* 4 (2.0%) respectively while *Proteus vulgaris*, Klebsiella spp and *Candida albicans* each comprised 2 (1.0%) while isolates from out-patients having *E. coli* constituting 15 (7.5%), *Staphylococcus aureus* comprising 9 (4.5%), *Proteus vulgaris* comprising 7 (3.5%) with Klebsiella spp and *Candida albicans* recording 6 (3.0%) respectively.

Cefuroxime and ceftazidime a second and third generation cephalosporin, fluoroquinolones (ofloxacin and levofloxacin), Augmentin and Amoxycillin were antibiotics used in this study. The *Candida albicans* isolated was not subjected to antibiotic susceptibility test as they do not respond to antibacterial agents.

Table 1: Percentage Prevalence of UTI among Diabetes mellitus Disease patients in Benin City, Edo State

Variable	No examined	Frequency	Percentage
Positive	200	57	28.5
Negative	200	143	71.5
Total	200		100

P<0.05

Table 2: Prevalence of UTI among Diabetes mellitus patients in Benin City, Edo State

Variable	No examined	No. positive	%age Positive	χ^2	p-value
Gender					
Male	62	10	17.5	6.748	0.009
Female	138	47	82.5		
Age (Years)					
<20	06	06	10.5	16.496	0.001
21 – 40	23	05	08.8		
41 – 60	120	30	52.6		
>60	51	16	28.1		
Educational Status					
Primary	34	17	29.8	9.354	0.009
Secondary	80	20	35.1		
Tertiary	86	20	35.1		

P<0.05

Table 3: Effect of some associated risk factors on the Prevalence of UTI among Diabetes milletus patients in Benin City, Edo State

Variable	No examined	No. positive	%age Positive	χ^2	p-value
Marital Status					
Single	08	05	08.8	4.727	0.030
Married	192	52	91.2		
Years infected with DM					
<5	84	18	31.6	7.565	0.023
6 – 9	104	32	56.1		
9 – 12	12	07	12.3		
Mode of Residence					
Rented	109	34	59.6	0.852	0.356
Own house	91	23	40.4		
Occupation					
Artisan	21	03	05.3	17.788	0.001
Civil servant	06	05	08.8		
Farming	04	02	03.5		
Self employed	157	40	70.2		
Retiree	12	07	12.3		

Table 4: Urinary tract pathogens Isolated among Diabetic mellitus Disease Patients

Organism Isolated	No examined	No. positive	%age Positive
Escherichia coli	200	19	33.3
Klebsiella sp	200	08	14.0
Proteus vulgaris	200	09	15.8
Staphylococcus aureus	200	13	22.9
Candida albicans	200	08	14.0
Total	200	57	100

Table 5: Distribution of Bacterial Species in relation to in-patient and out-patient Diabetes milletus patients in Benin City, Edo State

Organism Isolated	In-patient (%)	Out-Patient (%)
Escherichia coli	04 (28.6)	15 (34.9)
Klebsiella sp	02 (14.3)	6 (14.0)
Proteus vulgaris	02 (14.3)	7 (16.3)
Staphylococcus aureus	04 (28.6)	9 (20.9)
Candida albicans	02 (14.3)	6 (14.0)
Total	14 (100)	43 (100)

Table 6: Antimicrobial Sensitivity Pattern of Gram Negative and Gram Positive Organisms Isolated from DM Patient

			Antibiotics						
Group of Bacteria	Isolates	Isolates No	CAZ	CRO	LVX	OFX	AUG	AML	NA
Gram Negatives	*E. coli*	19	S-12(S) R-7(R)	9(S) 10(R)	8(S) 11(R)	7(S) 12(R)	8(S) 11(R)	7(S) 12(R)	6(S) 13(R)
	Klebsiella spp	8	S-2(S) R-6(R)	1(S) 7(R)	1(S) 7(R)	1(S) 7(R)	0(S) 8(R)	0(S) 8(R)	1(S) 7(R)
	Proteus vulgaris	9	S-9(S) R-0(R)	9(S) 0(R)	1(S) 8(R)	0(S) 9(R)	1(/S) 8(R)	9(S) 0(R)	0(S) 9(R)
Gram Positive	*Staphylococcus aureus*	13	S-13(S) R-0(R)	13(S) 0(R)	13(S) 0(R)	13(S) 0(R)	3(S) 10(R)	0(S) 13(R)	- -

KEY: S - Sensitive, R - Resistant, OFX - Ofloxacine, AUG - Augmentine, CAZ- Ceftazidime AML – Amoxicillin, CRO – Cefuroxime, NA - Nalidixic Acid, LVX - Levofloxacine

DISCUSSION

This study recorded a prevalence of 28.5% urinary tract infection among diabetic mellitus patients. This result is similar to a study by Foxman (2002) who observed 26% of UTI among DM patients in Washington and commonly reported prevalence of UTI that ranged from 8% - 26% in diabetic patients (Sherwin and Jastreboff, 2012; Bartoli and Ettare, 2014). UTI prevalence in females was 34.1% when compared with their male counterparts (16.1%). Our result agrees with previous studies that recorded UTI is significantly higher in female DM patients (47.9%, 88.5%, 7.2%) than male DM patients (34.1%, 11.5%, 4.7%) (Janifer *et al.,* 2009; Saleem, and Daniel, 2011; Ruhembe *et al.*, 2014). Age group 41-60years recorded the highest percentage prevalence (52.6%), followed by age group >60 years (28.1%), then age group <20 years (10.5%) with age group 21-40 years recording the least prevalence (8.8%).This result is similar to that obtained from the study where UTI was higher in age group 41-50 years (28.6%) with age group 51-60years recording 17.2% (Ruhembe *et al.*, 2014) and an American study performed on a health service data base with more than 70,000 patients that reveals UTI increases with age among DM patients (Liu *et al.,* 2009). In our study, urinary tract infection was higher in those with secondary and Tertiary education (35.1% respectively) while those having primary education had the least prevalence (29.8%). This is contrary to the result obtained in another study where those in tertiary education had the highest prevalence (69.4%) followed by those with primary education recording 31.7% while secondary education recorded the least prevalence (21.7%) (Ruhembe *et al*., 2014).

DM patients infected with DM between 6-9years had higher prevalence (56.1%) followed by <5years DM patients (31.6%) with 9-12years DM patients recording the least prevalence (12.3%). Our result agrees with the fact that in diabetic patients, specific risk factors for UTI are usually the duration of diabetes and the presence of long term complications (Geerlings *et al.,* 2002)**.** Married participants recorded higher prevalence (91.2%) than their single counterparts (8.8%).Our study revealed that UTI prevalence among participants dwelling in rented apartments was higher (59.6%) than those residing in their own houses (40.4%). Self employed DM patients showed a higher prevalence of 70.2% followed by Retiree 12.3%, while those involved in Farming had the least prevalence (3.5%). Marriage, Mode of residence and occupation are known risk factors for the acquisition of UTI among diabetic mellitus patients (Geerlings *et al.*, 2002).

E.coli (33.3%) and *Staphylococcus. aureus* (22.9%) were the most predominant bacteria isolated from DM patients. This is in agreement with other studies elsewhere in Africa where *E. coli* and *Staphylococcus aureus* are the commonly isolated bacteria with a prevalence of 40-46% for *E. coli* and 15%-31.4% for *Staphylococcus aureus* (Geerlings *et al.*, 2002; Geerlings, 2008; Odoki *et al*., 2019). This study also recorded the presence of other Enterobacteriaceae such as *Proteus vulgaris* (15.8%) and Klebsiella spp (14.0%). Other studies recorded 14.3% *Klebsiella pneumoniae* and 20% Proteus spp (Goswami *et al.,* 2001; Geerlings, 2008). Out-patients in our study recorded higher prevalence of UTI (75.4%) than in-patients (24.4%). This is contrary to that obtained in another study where in-patients recorded higher prevalence of UTI (57.0%) among DM patients than out-patients (43.0%) (Odoki *et al.,* 2019). *Candida albicans* recorded 14.0% in our study. This is contrary to that obtained by Bamnote *et al.* (2018) that recorded 2.85% *Candida albicans* infection among DM patients in their study.

Cefuroxime and ceftazidime, a second and third generation cephalosporin had minimal inhibition against the Gram negative bacteria but had highest inhibition activity against the Gram positive bacteria isolated. The Gram negative bacteria isolated were not readily susceptible to the fluoroquinolones (ofloxacin and levofloxacin) but the Gram positive bacterium (*Staphylococcus aureus*) was susceptible to the fluoroquinolones. All the bacteria isolates obtained in this study were resistant to Augmentin and Amoxycillin. This is contrary to the result obtained by Bamnote *et al.* (2018) that observed Gram positive bacteria isolated showed significantly higher resistance rates to CIP, CTX, CAZ, GEN and NIT.

CONCLUSION

UTI prevalence obtained was 28.5%. Educational status, marital status, number of years infected with DM and occupation significantly influenced the acquisition of UTI among DM patient. The Gram negative bacteria were less inhibited by the cephalosporins and fluoroquinolones. The Gram positive bacterium was susceptible to the cephalosporins and resistant to the fluoroquinolones.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors Contributions

Laboratory investigations and statistical analysis were performed by Emoregan Avwerosuoghene Sarah assisted by Mr Igunma Aimuanmwosa Andrew. Moses-Otutu Ifueko Mercy designed the study and drafted the manuscripts. All the authors have read and approved the manuscript.

Acknowledgements

The authors will like to thank Mr Igunma Aimuanmwosa Andrew for his contributions in the laboratory and statistical analysis.

Funding Statement

This research was funded by the authors

REFERENCES

American Diabetes Association. Position statement. Standards of medical care in diabetes (2012). *Diabetes Care* 35: 11-63.

Bamnote, P. T., Ovhal, R. S., Kulkarni, D. M. and More, S. K. (2018). Prevalence of Urinary Tract Infections and its antibiogram in diabetic and non-diabetic patients attending tertiary care hospital in Maharashtra. IP International Journal of Medical Microbiology and Tropical Diseases 4(2): 76-79.

Bartoli, L. and Ettare, F. (2014). Fibrinogen plasma levels as markers for thrombin activation in diabetes. *Diabetes,* 3: 808 – 812.

Bonadio, M., Costarelli, S., Morelli, G. and Tartaglia, T. (2006). The influence of diabetes mellitus on the spectrum of uropathogens and the antimicrobial resistance in elderly adult patients with urinary tract infection. *BMC Infectious Diseases* 6: 54-55

Cheesbrough, M. (2006). Blood Collection. *District Laboratory Practice in Tropical Countries; Part 2.* 2nd ed. Cambridge University Press New York. Pp. 434-436

Chin-Hong, P. V. (2006). Infections in patients with diabetes mellitus: Importance of early recognition, treatment and prevention. *Advanced Studies in Medicine* 6: 71-81.

De Lastours, V. and Foxman, B. (2014). "Urinary tract infection in diabetes: epidemiologic considerations," *Current Infectious Disease Report* 16(1): 389-390.

Foxman, B. (2002). Epidermiology of urinary Tract Infections: Incidence, Morbidity and Economic Costs. *American Journal of Medicine* 113(1): 5-13

Geerlings, S.E. (2008). Urinary tract infections in patients with diabetes mellitus: epidemiology, pathogenesis and treatment. *International Journal of Antimicrobial Agents*. 31(1): 54 – 57.

Geerlings, S.E., Meiland, R., van Lith, E.C., Brouwer, E.C., Gaastra, W. and Hoepelman, A.I. (2002). Adherence of type 1-fimbriated *Escherichia coli* to uroepithelial cells: more in diabetic women than in control subjects. *Diabetes Care*. 25(8): 1405 – 1409.

Goswami, R., Bal, C. S., Tejaswi, S., Punjabi, G. V., Kapil, A. Kochupillai, N. (2001). Prevalence of urinary tract infection and renal scars in patients with diabetes mellitus. *Diabetes Research and Clinical Practice* 53: 181-186.

Janifer, J., Geethalakshmi, S., Satyavani, K. and Viswanathan, V. (2009). Prevalence of lower urinary tract infection in South Indian Type 2 diabetic subjects. *Indian Journal of Nephrology* 19(3): 107-111

Johnson, K. M., Ptaszynska, A., Schmitz, B., Sugg, J., Parikh, S. J. and List, J. F. (2013)."Urinary tract infections in patients with diabetes treated with dapagliflozin, *Journal of Diabetes and its Compli cations* 27(5): 473–478

Liu, F., Chen, G., Huo, Wenqian, Wang, Chongjian., Liu, Suyang., Li, Na., Mao, Shuyan., Hou, Yitan, Lu, Yuanan and Xiang, H. (2009). Associations between long-term exposure to ambient air pollution a d risk of type 2 diabetes mellitus: A systematic review and meta-analysis *Environmental Pollution* 252: 1235-1245

Mnif. M. F., Kamoun, M., Kacem, F. H., Bouaziz, Z., Charfi, N., Mnif, F., Naceur., B. B., Rekik, N. and Abid, M. (2013). Complicated Urinary Tract Infections associated with diabetes mellitus: Pathogenesis, diagnosis and management *Indian Journal of Endocrinology and Metabolism* 17(3): 442-445

Odoki. M., Aliero, A. A., Tibyangye, J., Maniga, J. N., Wampande, E., Kao, C. D., Agwu, E. and Bazira, J. (2019). Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda. *International Journal of Microbiology* 1:1-9

Oslen, B.E., Hunderaker, S.G. and Lie, R.T. (2000). The diagnosis of urinary tract infection among pregnant women in rural Tanzania, prevalence and correspondence between different diagnostic methods. *Acta Obstetricia et Gynecologica Scandinavica* 79: 729-736.

Ruhembe, C. C., Mosha, C. E. T. and Nyaruhucha, C. N. M. (2014). Prevalence and awareness of type 2 diabetes mellitus among adult population in Mwanza City, Tanzania. *Tazania Journal of Health Research* 16(2): 1-11

Saleem, M. and Daniel, B. (2011). "Prevalence of urinary tract infection among patients with diabetes in Bangalore city," *International Journal of Emerging Science* 1(2): 133–142.

Sherwin, R. and Jastreboff, A. M. (2012).Year in Diabetes: The Diabetes Tsunami. *Journal of Clinical Endocrinology and Metabolism* 97(12): 4293-4301

Vasudevan, R. (2014). Urinary tract inf ection: An overview of the infection and the associated risk factors. *Journal of Microbiology and Experimentation,* 1 (2): 42-54.