Research Article

Detection of Asymptomatic Bacteriuria and Antimicrobial Susceptibility Testing among Diabetic Patients in Khartoum State

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Abstract:

Background: Urinary tract infections (UTIs) are one of the most common infections seen in all age groups with diabetes mellitus (DM). The term asymptomatic bacteriuria (ASB) refers to the isolation of bacteria in a urine specimen of individuals with unobserved symptoms of UTIs. DM is one of the risk factors of UTIs and causes complications including renal abscess, cystitis, fungal infections, pyelonephritis, and renal papillary necrosis. ***Objectives***: This study aimed to detect asymptomatic bacteriuria and antimicrobial susceptibility patterns for isolated organisms among adult, asymptomatic diabetic patients were attended selected diabetic hospitals and centers in Khartoum state. ***Methods***: A descriptive, prospective cross-sectional study was conducted on 120 asymptomatic diabetic patients from July to October 2022 in Khartoum state, Sudan. Information about patient demographics and clinical status was obtained from each patient using a written questionnaire. Clean-catch midstream urine specimens were collected and cultured on CLED, then processed for isolation and identification of uropathogens through conventional microbiological procedures. Antibiotic susceptibility patterns were determined by using the Kirby-Bauer disc diffusion method through culturing the isolates on Mueller- Hinton agar. The collected data and laboratory results were analyzed using SPSS version 26. ***Results***: 120 asymptomatic diabetic patients were included in this study, the age -mean was 44.8±11.76, out of which 16.7% (n=20/120) showed significant ASB and 75%(n=15/20) of them were females. In this study, there was a significant association between the level of HbA1c (*P. value* 0.049), bacteriuria (*P. value* 0.000), and ASB among studied diabetic patients, on the other hand, no significant association between age, gender, or type of DM, duration of DM, recurrent UTIs, other study variables and ASB. *S. aureus* was the commonest isolated uropathogen (40%) followed by *P. aeruginosa* (25%), *E. coli* (15%), *E. faecalis* (15%), *C. koseri* (5%), *S. aureus* isolates were resistant to oxacillin in (62%). The isolated organisms were resistant to cefotaxime (50%), gentamycin (50%), imipenem (35%), nalidixic acid

(75%) ciprofloxacin (40%). *Conclusion*: The overall prevalence of ASB among asymptomatic diabetic patients was high (16.7%). In this study poor glycemic control is a significant risk factor for ASB**.** ***Conclusion*****:** Regular screening for ASB through culture and antimicrobial susceptibility testing is recommended mainly for females over 45 years.

Keywords: Bacteriuria, Diabetic Patients, Asymptomatic, Antimicrobial Susceptibility Testing, Sudan.

Introduction:

Urinary tract infections (UTIs) are a group of common diseases that are mostly caused by the ascension of normal enteric flora through the urethra into the bladder these infections more frequently affect women because of anatomic differences **[1].** UTIs are one of the most common infections seen in all age groups with diabetes mellitus (DM). Susceptibility to urinary tract infection among diabetic patients is very high compared to non-diabetic **[2].** Host immune system abnormalities due to DM such as impaired migration, chemotaxis phagocytosis, and intracellular killing potential of polymorphonuclear cells, and local complication related to neuropathy like impaired bladder emptying and higher glucose concentration of urine in diabetic patients enhance UTIs. Patients with DM have a higher prevalence of asymptomatic bacteriuria (ASB) and a higher incidence of symptomatic UTIs, which more often lead to compared with those without DM **[3].** The reason for the greater frequency of infections in DM patients includes incompletely defined abnormalities in cell-mediated immunity and phagocyte function associated with hyperglycemia as well as diminished vascularization. Pneumonia, urinary tract infections (UTIs), and skin and soft tissue infections are all more common in the diabetic population **[4].** With this disease on the rise, diabetes mellitus has become a hot topic of discussion ultimately leading to further elaboration of disease processes that can ensue due to its initial ailment of it. Diabetes mellitus is notorious for causing cardiovascular, neurological, and renal insult **[5,6].** Moreover, UTIs are associated with end-stage renal disease or impaired renal function among pediatric patients, leading to several abnormalities in patients with an increased risk of pyelonephritis, increased premature delivery, and high fetal mortality among pregnant women **[7-9].** In general, DM can increase the level of urine glucose and pH, so the urine becomes an appropriate microenvironment for harmful bacteria to grow and reproduce **[10].** Adequate metabolic control not only limits complications of the disease but also lowers the risk of acquiring infection in an already susceptible diabetic patient **[11].** Type-2 diabetes mellitus (T2DM) increases the risk of many infections. the urinary tract is the most common location for this infection which occurs in part due to associated immune and nervous system defects caused by hyperglycemia and partly by glucose-rich environment (glycosuria) in the urinary tract infection**[12,13].** Which can produce more serious outcomes in patients with DM **[14,15].** There is the consensus is that most uropathogenic microorganisms such as Escherichia coli colonize the colon and in females the entrance to the vagina and the area around the urethra **[16].** ASB is known as the presence of significant bacteriuria without the symptom of an acute UTI **[17].** ASB diagnosis in women was based on the presence of a colony count of ≥105 CFU/ml of the same bacterial strain documented on

two consecutive samples within two weeks in the absence of urinary tract infection symptoms, on the other hand; asymptomatic bacteriuria in men was defined as a colony count of ≥105CFU/ml documented once in an asymptomatic person **[18].** UTIs are caused by the colonization and growth of microorganisms such as bacteria, fungi, and viruses **[19,20].** The two latter are the least cause of UTIs **[21].** The primary etiological agents of urinary tract infection are Gram-negative bacteria; among which *Escherichia coli* is the most common cause of UTI in men and women with and without DM **[22].** However Gram-positive bacteria may also be involved in UTIs **[23].**

Materials and methods:

Study design:

A descriptive, prospective cross-sectional study.

Study area:

The study was conducted at selected hospitals in Khartoum, these include Diabetes Hospital (Khartoum North), and Gaber Aboalez Diabetic Care Hospital (Khartoum).

Study duration:

This study was carried out from July to October 2022.

Study population:

Diabetic patients in Khartoum state.

Sampling type:

Non- probability, convenience sampling technique.

Sample size:

One hundred and twenty asymptomatic diabetic patients were enrolled in our study. Was calculated by using the Raosoft sample size software calculator at a confidence interval (CI) of 95%, and a margin of error of 8.92 %.

Data collection tools:

Any participant was taught the information to collect clean-catch midstream urine in a sterile urine container. A written questionnaire was used to collect socio-demographic data (age, gender), medical information data; type of diabetes, duration of diabetes, HbA1c status, hypertension, history of UTI, chronic kidney disease, recurrent UTIs, prostate problems, hemodialysis, and cigarette smoking.

Specimen collection:

The urine specimens were collected in sterile urine containers and stored in an ice box at 4°C until they were cultured.

Equipment and instrument:

Light-field microscope, incubator, autoclave, hot air oven, sensitive balance, sterile urine containers, sterile disposable calibrated loops (1.0-10.0µl), sterile plastic Petri dishes (90mm), slides, sterile test tubes, sterile cotton swabs, glass bottles, flasks, cylinders, wooden sticks, centrifuge, Bunsen burners. An autoclave was used for the sterilization of culture media, a hot air oven was used for the sterilization of glass wares, and drying of culture media. Incubator was used to incubate the microorganisms aerobically at 37°C overnight, the sensitive balance was used to weight powders of culture media and chemicals, a centrifuge, and a light Microscope were used for urine examination

and indirect Gram's stain examination, flasks and bottles were used for media preparation and heater were used for dissolving culture-media powders.

Data analysis:

Statistical analysis of the data was performed using Statistical Package for the Social Sciences (SPSS) software, version 26.

Ethical consideration:

The study clearance was obtained from the ethical committee of Alfarj College of Sciences and Technology, and the Medical Laboratory Sciences Program. Departmental permission was obtained from different hospitals. Verbal consent was taken from each participant before the collection of specimens.

Results:

In our study, the total number of participants was 120 asymptomatic diabetic patients for UTIs, (41.7%) were males and the majority (58.3%) were females. The age mean/ years was (44.8 ±11.76), the minimum age was 20 years and the maximum was 60 years, the age -group distribution (41.7%) of the participant-age was > 50 years (**Table 1**). In our study; (53%) of the patients had type 1 diabetes, while (47%) had type 2. (55%) of them had a normal level of HbA1c and (45%) had a high level of HbA1c which is considered uncontrolled diabetes. The duration of diabetes was; <1 year (29.5%), 1-5 years (36.7%), 6-10 years (5%), and > 10 years (29.2%). A history of hypertension was found in (26.7%) of the patients, and none of them (0.0%) had a history of chronic kidney disease, hemodialysis, recurrent UTIs, and prostate problems, but (4.2%) of the males were cigarette smokers (**Table2**). Dipstick strips were used to screen the urine specimens of diabetic patients for UTIs. The results of these tests were; (100.0%) of the urine specimens were acidic, glucose was positive in (40.8%), leukocyte esterase was positive in (13.3%), nitrate reductase was positive in (12.5%), total pus cells were detected in (85.8%) at different numbers. RBCs also were detected in (66.6%), and bacteria were found in (13.3%) of diabetic urine specimens (**Table3**). In this study the overall prevalence of significant ASB among diabetic patients was (16.7%, n= 20/120). Among all isolated bacteria (n=20); *S. aureus* was the predominant isolate (40%) followed by *P. aeruginosa* (25%), *E. coli* (15%), *E. faecalis* (15%) and *C. koseri* (5%), (75%) (n=15/20) of the infected diabetic patients were females, and (75%) of *S. aureus* isolates were isolated from females, and all *E. faecalis* isolates (n=3) were isolated from females; so, in our study, the females were infected by Gram-positive bacteria more than Gram-negative bacteria (**Table4**).Among the cultures that returned positive (n= 20), *S. aureus* (n=8) was tested against oxacillin to detect MRSA and the percentage was (62.5%). For cefotaxime, overall isolated bacteria; (50%) were resistant, (15%) showed intermediate sensitivity, and (35%) were sensitive to cefotaxime. For gentamycin (50%) of all isolates were resistant and (50%) were sensitive. For imipenem, and nalidixic acid, resistant percentages were (35%), (and 75%)

respectively, while sensitivity percentages were (65%), and (25%) respectively. For ciprofloxacin (40%) were resistant, (5%) showed intermediate sensitivity, and (55%) were sensitive. In conclusion, imipenem showed the least resistance percentage (35%), while nalidixic acid showed the highest resistance percentage (75%). Antibiotic-resistance patterns of isolated bacteria to various tested antibiotics. *S. aureus* isolates showed (62.5%) resistance to oxacillin and it is considered MRSA. (100%) of *P. aeruginosa* isolates were resistant to nalidixic acid while (60%) were resistant to cefotaxime. However, (100%) of the isolated *E. coli* were resistant to all tested antibiotics so we considered it as multi-drug resistant (MDR). *C. koseri* showed the least resistance to Antibiotics may be because its number was few in our study (**Table5**). The overall prevalence of significant ASB was (16.7%), despite females being most infected (15/20) than males but statistically, there was no association between gender and significant ASB (positive culture) (*P. value* 0.098) (**Table6**). In this study, we found a significant association between significant ASB and HbA1c levels (*P. value* 0.049). Conversely, there was no association between, age group, type of diabetes, duration of diabetes, and significant ASB, (*P. value* 0.939), (*P. value* 0.740), (*P. value* 0.932) respectively. Among urine screening tests, the only significant correlation was found between significant ASB and bacteriuria (*P. value* 0.000) In conclusion; the result findings of this study showed there was a correlation between HbA1c level, bacteriuria, and significant ASB (**Table7**).

Table-1: Distribution of age- group of the participants.

Age- group/years	*Frequency*	*Percent (%)*
20-29	16	13.3
30-39	20	16.7
40-49	34	28.3
>50	50	41.7
Total	**120**	**100.0**

Table-2: Distribution of clinical characteristics of diabetic patients.

Clinical characteristic	*Status*	*Frequency*	*Percent%*
Hypertension	*No*	88	73.3
	Yes	32	26.7
	Total	120	100
Chronic kidney disease	*No*	120	100
	Yes	0	0
	Total	120	100
Hemodialysis	*No*	120	100
	Yes	0	0
	Total	120	100
Recurrent UTIs	*No*	120	100
	Yes	0	0
	Total	120	100
Prostate problems	*No*	120	100
	Yes	0	0
	Total	120	100
Cigarette smoking	*No*	115	95.8
	Yes	5	4.2
	Total	120	100

Table-3: Distribution of urine screening tests.

Urine screening test	*Result*	*Frequency*	*Percent %*
Glucose	*Negative*	71	59.2
	Positive	49	40.8
	Total	**120**	**100.0**
pH	*Acidic*	120	100.0
	Alkaline	0	0.0
	Total	**120**	**100.0**
Lecukocyte estrase	*Negative*	104	86.7
	Positive	16	13.3
	Total	**120**	**100.0**
Nitrate reductase	*Negative*	105	87.5
	Positive	15	12.5
	Total	**120**	**100.0**
Pus cells/ PF (Pyuria)	*1-5*	84	70.0
	6-10	16	13.3
	>10	3	2.5
	Negative	17	14.2
	Total	**120**	**100.0**
RBCs / HPF	*1-5*	76	63.3
	6-10	3	2.5
	>10	1	0.8
	Negative	40	33.3
	Total	**120**	**100.0**
Bacteria	*Negative*	104	86.7
	Positive	16	13.3
	Total	**120**	**100.0**

Table-4: Distribution of isolated bacteria from asymptomatic diabetic patients.

Isolated bacteria	*Frequency*	*Percent %*
S. aureus	8	40
Female	(6)	(75)
Male	(2)	(25)
P. aeruginosa	5	25
Female	(3)	(60)
Male	(2)	(40)
E. coli	3	15
Female	(2)	(66.7)
Male	(1)	(33.3)
E. faecalis	3	15
Female	(3)	(100)
male	(0)	(0)
C. koseri	1	5
Female	(1)	(100)
Male	(0)	(0)
Total	**20**	**100.0**

Table-5: Antibiotic- resistance patterns of isolated bacteria.

Antibiotic / *Isolate*	OX R (%)	CTX R (%)	CIP R (%)	IMP R (%)	CN R (%)	NA R (%)
S. aureus(n=8)	62.5	37.5	25	25	50	37.5
P. aeruginosa (n=5)	-	60	40	40	40	100
E. coli(n=3)	-	100	100	100	100	100
E. faecalis(n=3)	-	33.3	33.3	0	33.3	33.3
C. koseri(n=1)	-	0	0	0	0	100

Table-6: Association of independent variables with significant ASB (positive culture) among diabetic patients.

Study variable	*Status*	*Negative*	*Positive*	*Total*	*P. value*
Gender	*Male*	45(45.0%)	5(25.0%)	50(41.7%)	0.098
	Female	55(55.0%)	15(75.0%)	70(58.3%)	
	Total	**100(100.0%)**	**20(100.0%)**	**120(100.0%)**	
HbA1c	*High*	41(41.0%)	13(65.0%)	54(45.0%)	0.049
	Normal	59(59.0%)	7(35.0%)	66(55.0%)	
	Total	**100(100.0%)**	**20(100.0%)**	**120(100.0%)**	
Type of diabetes	*Type 1*	54(54.0%)	10(50.0%)	64(53.3%)	0.740
	Type 2	46 (46.0%)	10(50.0%)	56(46.7%)	
	Total	**100(100.0%)**	**20(100.0%)**	**120(100.0%)**	
Duration of diabetes/year	20-29	13 (13.0%)	3(15.0%)	16(13.3%)	0.932
	30-39	18(18.0%)	2(10.0%)	20(16.7%)	
	40-49	27(27.0%)	7(35.0%)	34(28.3)	
	Total	**100(100.0%)**	**20(100.0%**	**120(100.0%**	

Table-7: Correlation between study variables and ASB among diabetic patients.

Study variable	*R*	*P value*
Age group	0.007	0.939
Gender	0.151	0.099
Type of diabetes	- 0.001	0.991
Hb A1c	- 0.180	0.049
Duration of diabetes	- 0.054	0.560
Bacteriuria (bacteria in urine)	0.482	0.000
Urine RBCs	-.125	0.175
U. pus cells	- 0.003	0.970
U. Leukocyte esterase	0.022	0.812
U. Nitrate	- 0.101	0.270

Discussion:

Diabetes mellitus has long been implicated as a predisposing factor for UTIs. Moreover, it is a well-established fact that the urinary tract is the primary site of infection in diabetic patients with an increased risk of complications of UTIs **[5].** The findings of the present study provided baseline information on the prevalence of ASB in diabetic patients, socioeconomic status, clinical characteristics, etiological profile, and antibiotic susceptibility patterns. In our study, the prevalence of ASB was 16.7%, which this higher than the results of two studies performed by Feleke Y *et al* (2007) and Yeshitela B *et al* (2012) in Addis Ababa Ethiopia in which diabetic patients were evaluated, the prevalence of ASB was reported as 10.4%,14% respectively **[24,25]**. On the other hand, a higher prevalence was detected by Simkhada R *et al* (2013) in Nepal at 21% **[26].** Hamdan Z *et al* (2015) in Khartoum-Sudan found the prevalence of ASB was 20.9% **[27].** The variation in the prevalence might be explained by the difference in geography, the host factor, and practices such as social habits of the community, standards of personal hygiene, and health education practices. In our study, there was an association between HbA1c, bacteriuria, and significant ASB the same as found by Bashir A *et al* (2021) in Srinagar. India in which high levels of HbA1c and bacteriuria were considered as main risk factors for ASB **[28].** The patient's age, gender, types of DM, and duration of DM, were not associated factors for ASB in our study, in contrast of the findings of Patterson JE *et al* (1997) and Hammar N *et al* (2010) they found there were associated with older age' duration of DM and level of DM control and were risk factors for UTI among diabetic patient **[29,30].** In this study urine screening tests leukocytes esterase, nitrate, pus cells, and red blood cells were not associated with significant ASB, this agreed with a study by Al- Rubeaan KA *et al* (2013) found none of the investigated factors were associated with the prevalence of UTIs **[31].** In this study the most predominant microorganism was *Staphylococcus aureus* (40%) this may be due to in our study, ASB was mainly found in females and S. *aureus* was normal flora in the vagina and perineum skin, followed by *Pseudomonas aeruginosa* 25%, *E.coli* 15%, *E. faecalis* (15%) and *C.koseri* (5%) this agreed with another study done by Odetoyin *et al* (2008) in Nigeria found the predominant organism was *S. aureus* 80.9% **[32].** Also this disagreed with another study done by Zhanel *et al* (1995) found the prevalence of *E. Coli* 52.9% but also isolated *Streptococcus* species (11.4%)and *staphylococcus* species 5.9% **[33].** In our study we found the *E. coli* isolates were resistant (100%) to all tested antibiotics: gentamycin, nalidixic acid, imipenem, cefotaxime, and ciprofloxacin, this result was agreed with another study done by Bisson *et al* (2013) in Cameroon, found that *E. Coli* was resistant to nalidixic acid (33.3%) and gentamycin 26.7% **[34].** Another study done by Nigussie D *et al* (2017) found *E. coli*

was resistant to gentamycin in 27.7% **[35].** In our study, the other isolated bacteria also exhibited resistance against similar antibiotics with varying degrees. Since the prevalence of resistance exhibited by the Gram-negative uropathogens against routinely used antibiotics is at high levels, it is a major setback for the effective management of UTIs.

Conclusion:

In this study, the overall prevalence of significant ASB among diabetic patients was (16.7%). A higher prevalence of ASB in diabetics patients was observed among females 15/20 (75%) more infected than males (25%). The most commonly identified bacteria were *S. aureus* (n=8) was the predominant isolate followed by *P. aeruginosa* (n=5), *E. coli* (n=5), *E. faecalis* (n=3), *C. koseri* (n=1), (75%) of *S. aureus* isolated from females, and they were mainly infected by Gram-positive bacteria (11/20) more than Gram-negative bacteria. Antibiotic-resistant patterns of isolated bacteria to various tested antibiotics, *S. aureus* showed (62.5%) resistance to oxacillin and it could be considered as MRSA, (and 100%) of *P. aeruginosa* isolated were resistant to nalidixic acid, while (60%) were resistant to cefotaxime. However, (100%) of the isolated *E. coli* were resistant to all tested antibiotics so we considered it as multi-drug resistant (MDR). *C. koseri* showed the least resistance to the antibiotic may be because its number was few in our study. The result findings of this study showed there was a correlation between HbA1c level, bacteriuria, and significant ASB.

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Conflict of Interest:

The author has affirmed that there are no conflicting interests.

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