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Original Article

Prevalence of Asymptomatic Bacteriuria among Patients Attending Diabetic Clinic at Fort Port Regional Referral Hospital, Uganda Patients

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Abstract

The availability of high concentration of sugar in the blood of diabetic patients makes them more susceptible to developing bacterial infections which are asymptomatic commonly referred to as asymptomatic bacteriuria (ASB) than the non-diabetic patients. Diabetes patients with ASB have a much higher risk of developing diabetic complications than individuals without ASB. There is a paucity of data on the burden of ASB and antimicrobial susceptibility in diabetes patients in Uganda. Between March and May 2023, a cross-sectional study was carried out at the Fort Portal Regional Referral Hospital, Uganda outpatient diabetic clinic and 160 diabetes patients were recruited for the study. Mid-stream urine was taken for culture and sensitivity. Data analysis were performed using IBM SPSS version 20. A pie chart was used to display the prevalence and antimicrobial susceptibility patterns of common uropathogens described in frequency and percentages. The overall prevalence of ASB among the study participants was 11%. The most common uropathogen isolated was *Escherichia coli*, followed by *Klebsiella* and *S. aureus*. The most sensitive drug in all the isolates was Nitrofrantoin and resistant drug was Ampicillin. In conclusion, routine testing for ASB and treatment should be according to the antimicrobial susceptibility of diabetic patients.

Keywords: Prevalence, Diabetes mellitus, Asymptomatic bacteriuria, Antibiotic susceptibility, Uropathogens.

INTRODUCTION

Diabetic mellitus is one of the metabolic diseases manifested in the form of high blood sugar that remains in the blood stream and cannot be pushed into the cells [1]. This result in high level of blood sugar above the recommended threshold in the body, a condition commonly described as hyperglycemia [1]. It is a disease that affects people of all ages and it is the 8th leading cause of death in the world. It is projected that the number of people living with diabetes will surpass 1.3 billion from the current 500+ million in the next 30 years [2]. Virtually, every country is suffering from the burden of managing diabetes mellitus from people of all ages.

Patients with DM experience a variety of polymorph nuclear abnormalities, including glycosuria, the inadequate immune response commonly known as neutrophil dysfunction, increased adhesion of uropathogens to epithelial cells, weakened innate and acquired immunity and bowel bladder syndrome are present [3]. The range of urinary tract infections (UTIs) in DM includes asymptomatic bacteriuria (ASB), cystitis, pyelonephritis, and severe urosepsis, and they can experience serious urinary tract infection (UTI) consequences more commonly than the general population, including emphysematous cystitis, pyelonephritis, renal abscesses, and renal papillary necrosis [4].

Diabetes patients have a prevalence of asymptomatic bacteriuria (ASB) that ranges from 9 to 27% or more, which is unquestionably greater than that of healthy people [5]. Several studies have revealed the presence of asymptomatic bacteriuria in various countries ranging from 11%-33% [6-9].

Common pathogens responsible for most cases of asymptomatic bacteriuria among diabetic patients, as well as their antimicrobial susceptibility patterns, may vary from region to region, which may

be caused by, among others, a variety of individual genetic differences, social, economic, personal hygiene, geographical differences, and ethnicity [10].

This study is aimed to investigate the presence of ASB in diabetic patients in Fort Portal Referral Hospital, Uganda. The findings of this study will be of great help for effective health care delivery and will go a long way in helping the policy makers to decide on the antibiotic to be used in the treatment of asymptomatic bacteriuria in this region.

MATERIAL and METHODS

Study Site

The study is a cross-sectional study which was conducted between 1st March 2023 to 1st June 2023 to determine the prevalence of asymptomatic bacteriuria and antimicrobial susceptibility patterns among diabetic patients attending Fort Portal Regional Referral Hospital (FPRRH) in western Uganda, which is located at Latitude: 0° 3910.79 N and Longitude: 30° 0° 16' 33.00. The hospital is one of the affiliated teaching hospitals for Kampala International University's Western Campus.

Study Population

A total of 160 diabetic patients attending for diabetic follow-up at the diabetic clinic of Fort Port Regional Referral Hospital, Uganda were included in the study.

Inclusion and Exclusion Criteria

Diabetic patients over 18 years of age who signed consent forms without symptoms of UTI were included in the study. Diabetic patients who took any antibiotics for the last 14 days during data collection, critical-ill patients, and pregnant women were excluded.

Data Collection

Patient's socio-demographic characteristics, medical history, and all required information were collected by questionnaire sheets, which were ethically approved. Each participant had 3 ml of fasting blood drawn into vacutainers. The level of blood glucose was determined for each specimen. Blood sugar was estimated to meet American Diabetic Association ADA criteria; fasting blood glucose (FBG) greater than or equal to 7.0 mmol/l or random plasma glucose ``level ≥ 11.1 mmol/l and hyperglycemic symptoms. Sample for asymptomatic bacteriuria (ASB) was collected according to Infectious Diseases Society of America (IDSA) criteria. ASB refers to the presence of 10^5 CFU/ml of one or more bacterial species in a urine sample from a person without clinical signs of a UTI.

Specimen collection

Every participant received instructions on how to fill a sterile, wide-mouthed, screw-capped container with 10 ml of clean midstream urine and how to avoid contamination. After collection, specimens were maintained at cold chain transportation using ice packs in a cool box. Upon arrival at the laboratory, all samples underwent quick analysis to guarantee the isolation of any pathogenic organisms found in the urine and to prevent any overpopulation of those organisms. The laboratory at Fort Portal Regional Referral Hospital (FPRRH) conducted the microbiological tests for this study.

Isolation and inoculation of uropathogens

The color, volume, turbidity, cloudiness, and odor of each urine sample were assessed, For asymptomatic bacteriuria, a colony count of 10^5 CFU/ml is deemed significant. Using a sterile standard calibrated wire loop (0.001 ml), urine specimens were immediately inoculated onto blood agar and MacConkey agar. Following a 24-hour aerobic incubation period at 36°C, the culture plates were checked for bacterial growth and their cultural features [6]. For asymptomatic bacteriuria, a colony count of 10^5 CFU/ml is deemed significant. The gram reaction, biochemical test, and colony morphology were used to identify the bacterial isolates[6].

Antimicrobial susceptibility tests

Commercially accessible antibiotic discs were used in the antimicrobial susceptibility testing (Kirby Bauer disk diffusion) method, which employed Muller- Hinton agar. The antibiotic tests were conducted using prepared Muller- Hinton agar media (Oxoid, Hampshire, UK). The 0.5 McFarland turbidity standard was used to modify the turbidity of the microorganisms that were going to be examined. The 0.5 McFarland turbidity standard was used to compare the isolated bacterial colonies

after they were moved to a dry and cleaned tube filled with sterile physiological saline and combined to create a homogenous solution. To make sure that the bacteria suspension was distributed evenly across the media surface, a sterile cotton swab was dipped into the suspension and pressed firmly against the inside wall of the tube. To enable the bacteria suspension to be absorbed into the media, the plates were let stand at room temperature for at least thirty minutes. For 18 to 24 hours, the plates were incubated at 37°C with the lids on. Following incubation, the antibiotics' sensitivity, resistance, and mild sensitivity to the tested bacteria were ascertained by measuring the zone of inhibition's diameter in millimeters using a ruler and comparing the results to a standard chart[7].

Quality control

To assess internal validity and reliability, a questionnaire was used that had been revised before it was used to ensure uniformity and clarity. Before utilizing the culture, the sterility and efficacy of the cultural media were assessed.Good clinical laboratory practice (GCLP) and standard operating protocols were followed in the analysis of all the samples.

Statistical analysis

The filled-out surveys were extracted and moved to Microsoft Excel 2019 for cleaning and coding. Data analysis is done using IBM SPSS version 20. Variables with a 95% confidence level were fitted with a P value of 0.2 into a multivariable logistic regression model to account for confounders. Numerical data were summarized using means, standard deviations, medians, and ranges, with P-values deemed statistically significant if they were less than 0.05. In IBM SPSS version 20, multivariate analysis has been utilized to assess correlations between independent and dependent variables, while frequencies and proportions have been employed to characterize categorical data.

RESULTS

Socio-demographic characteristics of the participants

A total of 160 patients were recruited and more than half of the participants, 102 (63.7%), were female; most of them resided in rural areas, accounting for 132 (82.5%), and had only completed primary school, or 86 (53.7%). 120 (75.0%) of them were unemployed. The mean age was 51.8 years, and the standard deviation was 1.1.

Table 1. Socio – demographic characteristics of the study participants (N = 160)

Variable	Category	Frequency (n)	Percentage (%)
Age	≤45 years	54	33.8
	>45 years	106	66.2
Mean age ±SD(years)	51.8±1.1		
Sex	Male	40	25.0
	Female	120	75.0
Residence	Rural	132	82.5
	Urban	28	17.5
Marital status	Single	32	20.0
	Married	102	63.7
	Divorced	6	3.8
	Widowed	20	12.8
Level of education	No formal	35	21.9
	Primary	86	53.7
	Secondary and higher	39	24.4
Employment status	Employed	40	25.0
	Not Employed	120	75.0

Prevalence of asymptomatic bacteriuria in the diabetic patients

A total of 160 patients were recruited, and 17 (11%) of them were found to have asymptomatic bacteriuria

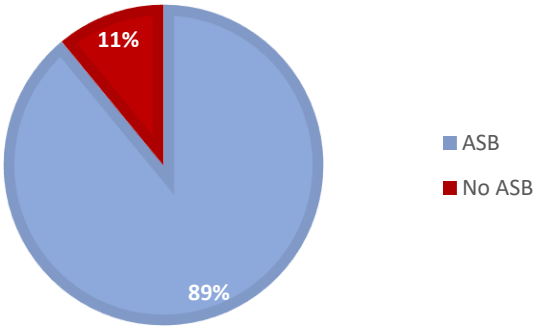

Figure 1. Prevalence of asymptomatic bacteriuria among diabetic patients attending the diabetic outpatient clinic at FPRRH

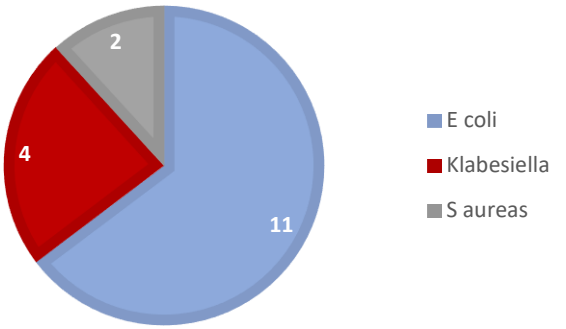

Figure 2. Common uropathogens in diabetic patients with ASB among diabetic patients attending diabetic outpatient clinic at FPRRH

Commonly isolated uropathogens

Escherichia coli was the most commonly isolated microorganism from ASB-positive patients, appearing in 11 out of 17 cases (64.7%), followed by *Klebsiella* sp (4 cases, 23.5%), and *Staphylococcus aureus* (2 cases, 11.76%).

Antimicrobial susceptibility pattern of isolated uropathogens

The bacteria E. coli show the maximum sensitivity to imipenem and nitrofurantoin, each scoring 11 (100%) and 9 (81.8%), respectively. Imipenem 3 (75%), then piperacillin 4 (100%), demonstrated the greatest sensitivity in *Klebsiella* sp. Cefotaxime, piperacillin, doxycycline, and ceftriaxone all exhibited 100% sensitivity in S. *aureus*.

Table 2. Antimicrobial susceptibility patterns of the common uropathogens in diabetic patients with ASB among people with diabetes attending diabetic outpatient clinic at FPRRH.

Sensitivity	Doxy	N/acid	Nitrof	Imip	Pipera	Cefo	Cef	Cotri	Amp	Cefur	Cip
E.coli (n=11)	8(72.7)	4(36.4)	11(100)	11(100)	9(81.8)	3(27.3)	2(18.2)	1(9.1)	0(0.0)	3(27.3)	3(27.3)
Klebsiella (n=4)	2(50)	2(50)	3(75)	3(75)	4(100)	1(25)	1(25)	3(75)	2(50)	2(50)	0(0)
S. aureus (n=2)	2(100)	1(50)	2(100)	1(50)	2(100)	2(100)	2(100)	0(0)	0(0)	0(0)	0(0)
Total (n=17)	12(70.6)	7(41.2)	16(94.1)	15(88.2)	15(88.2)	6(35.3)	5(29.4)	4(23.5)	2(11.8)	5(29.4)	3(17.6)

E. *coli=Eschericia coli*, S. *aureus=Staphylococcus aureus*, Doxy=Doxycycline, N/acid=Nalidixic acid, Nitrof=Nitrofurantoin, Imip=Imipenem, Pipera=Piperacillin, Cefo=cefotaxime, Cef=ceftriaxne, Cotri=Cotrimxazole, Amp=Apicillin,,Cefur=Cefuroxime, Cip=Ciprofloxacin.

Antimicrobial resistance of isolated uropathogens

The most resistant bacteria were E. *coli* to ampicillin (11 100%); *Klebsiella* sp to ceftriaxone (3 75%); and S. *aureus* to ciprofloxacin, cotrimoxazole, ampicillin, and cefuroxime (2 100%).

Table 3. Antimicrobial resistance test

Sensitivity	Doxy	N/acid	Nitrof	Imip	Pipera	Cefo	Cef	Cotri	Amp	Cefur	Cip
E.coli (n=11)	3(27.3)	7(63.6)	0(0.0)	0(0.0)	2(18.2)	8(72.7)	9(81.8)	8(72.7)	11(100)	8(72.7)	8(72.7)
Klebsiella (n=4)	2(50)	2(50)	1(25)	1(25)	0(0)	3(3)	3(75)	1(25)	2(50)	2(50)	4(50)
S. aureus (n=2)	0(0)	1(50)	0(0)	1(50)	0(0)	0(0)	0(0)	2(100)	2(100)	2(100)	2(100)
Total (n=17)	5(29.4)	10(58.8)	1(5.9)	2(11.8)	2(11.8)	11(64.7)	12(70.6)	11(64.7)	15(88.2)	12(70.6)	14(82.4)

E.*coli=Eschericia coli*, S. *aureus=Staphylococcus aureus*, Doxy=Doxycycline, N/acid=Nalidixic acid, Nitrof=Nitrofurantoin, Imip=Imipramine, Pipera=Piperacillin, Cefo=cefotaxime, Cef=ceftriaxne, Cotri=Cotrimxazole, Amp=Ampicillin,,Cefur=Cefuroxime, Cip=Ciprofloxacin

DISCUSSION

Diabetes mellitus (DM) is a syndrome characterized by impaired metabolism and excessive hyperglycemia brought on by either insufficient insulin secretion or a combination of insulin resistance and insufficient insulin secretion to compensate for the resistance. ASB is one of the consequences that increase morbidity and mortality. Polymorphic nuclear abnormalities include poor migration, phagocytosis, intracellular

death, and chemotaxis in patients with diabetes mellitus, such as hyperglycemia and host immune system deregulations encourage asymptomatic bacteriuria [11, 12].

According to the current study, asymptomatic bacteriuria was found in 11% (95%CI: 9.8-14.2%) of diabetes patients at Fort Portal Regional Referral Hospital, Uganda. The results of this investigation were comparable to those of a study done in Kenya by [13], where the prevalence of ASB was 11.1%. Moreover, 11.9% was reported in Ethiopia by [14]. The similarities between these researches could be explained by our common geographic location, ethnic background, and the fact that both were cross-sectional.

Our prevalence was significantly higher than that of [15], who found that the prevalence of ASB in DM was 10.3%. Furthermore, 8.1% was reported as the prevalence of ASB in Pakistan [16]. A research by [17] in China found that the lowered prevalence of ASB in DM was 3.7%.The variation could be accounted for by the following: more variety patient types with a wider range of characteristics, social life, social and economic origins, and geographic and ethnic backgrounds.

Contrary to what we found, several investigations reported a high prevalence of ASB. According to a study conducted in Pakistan, the prevalence of ASB was found to be 28.6%. This greater prevalence may have resulted from the diagnostic procedure used for ASB, which uses a combination strip to detect albumin and nitrates in urine. As a result, the prevalence of ASB can vary depending on the methodology used [17]. According to [8, 18], the percentages in India were 33.3% and 28.2%, respectively while in Cameroon, the percentage was 38.3% [22]. These studies were carried out in various geographic locations, which may have contributed to the observed variations.

Variations in sample size, culture or screening technique, demographic characteristics like cleanliness, region or environmental factors, health education methods, and genetic polymorphisms can all account for the variation in prevalence. People with diabetes have a weakened immune system, which leaves them more susceptible to many diseases as well as a range of polymorphic nuclear defects, such as chemotaxis, phagocytosis, intracellular death, and poor migration [11]. As high renal parenchymal glucose levels foster an environment that is favorable to bacterial growth and multiplication, they may be one of the risk factors for pyelonephritis and renal consequences such as emphysematous pyelonephritis [19].

According to our analysis, the most prevalent infections were found to be *E. coli* 11 (64.7%), *Klebsiella* 4 (23.5%), and *S. aureas* 2 (11.76%). This corresponds to studies conducted in Pakistan by [16], India by [5], and Nigeria by [10]. These study reports showed gram-negative *Bacilli*, with *E. coli* and *Klebsiella* sp receiving scores of 16 (36.4%) and 6 (13.6%), respectively. It was found that E. *coli* is the most common bacterial uropathogen in Western Uganda among several patient populations, including hospitalized, catheterized, HIV-positive, neonates, and pregnant women. This could be due to similar geographic location, ethnic population, environment, social customs of the community, standard of personal hygiene, and economic level; this could be related to our results [20]. But our results differed from those of in Sudan, where S. *aureus* (40%), then P. aeruginosa (25%), was the most prevalent isolate [21]. Variation was also noted in the results of [22] in Cameroon, where the most commonly isolated bacteria were *Klebsiella* sp and coagulase-negative *staphylococci*. There may be differences in the incidence of bacterial UTI etiologies between countries depending on the techniques used, the environment, and the social customs of the community, the education level, and the standard of personal hygiene.

Our susceptibility findings were notably consistent with those stated in a southern Nigerian hospital-based study conducted by [10], [6] in Nepal, [16] in Pakistan, and [14] in Ethiopia, where nitrofurantoin and imipenem were the most dominant antibiotics as regards sensitivity to the isolated bacteria. The results indicated good sensitivities, which might be explained by nitrofurantoin being prescribed less frequently. Bacteria also did not develop resistance mechanisms or molecular modifications, similar to our work. [14] in Ethiopia reported that ampicillin led to high levels of bacterial resistance. This is due to the fact that penicillin is cheap, widely accessible,

and inappropriately used to treat a variety of illnesses, including respiratory infections. Drug resistance is influenced by non-prescription drug use, which produces beta-lactamases.

We observed a disagreement with the results by [22] in Cameroon, where ciprofloxacin was the most effective antibiotic and had substantial resistance to cotrimoxazole, nitrofurantoin, and oxacillin.The development of beta-lactamase, which is linked to penicillin resistance may be responsible for this. The high level of resistance in this study area may be attributed to the frequent use of cotrimoxazole to treat UTIs and other infectious illnesses. [14] in Ethiopia found that amikacin and tobramycin were the most sensitive, as opposed to our study. This may be due to infrequent use of these drugs and thus result in high sensitivity.

CONCLUSION

The prevalence of asymptomatic bacteriuria in diabetic patients at FPRRH was 11%, where Escherichia coli is the most common uropathogen. The most sensitive drug across all the isolates was found to be Nitrofurantoin while the resistant drug was found to be Ampicillin. Appropriate empirical therapy for ASB can be determined by analyzing the antibiotic sensitivity patterns of commonly isolated pathogens. Implementation on time to minimize complications, including upper urinary tract infections and pyelonephritis.

Conflict of Interest

The authors declared that they have no conflict of interests.

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