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Asymptomatic Bacteriuria and Antibiogram of Isolates Among Diabetic Patients in Calabar, Nigeria

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Abstract

Asymptomatic bacteriuria is the presence of bacteria in the urine of an individual without any symptom of urinary tract infection. This has been widely observed in diabetic patients and could be detrimental to their health if not effectively managed. This study was to identify the pathogens associated with asymptomatic bacteriuria among patients with diabetes and the antibiogram of those isolates in Calabar. Blood samples were collected for the determination of fasting blood sugar levels using glucometer. Bacterial isolations were done through urine culture and antibiogram were tested in all urine samples of the diabetic patients in this study. The prevalence of bacteriuria in this study was 26.0%. Participants aged, 41-50 years were highest both in blood sugar level (12.3+/-4.38mmol/L) and infection rate (37.5%, 18/48). Females had lower blood sugar (10.9+/-3.370mmol/L) than the males (11.3+/-4.46mmol/L) while the males had less infection rate (22.4%, 22/98) than the females (29.4%, 30/102). *Escherichia coli*, 46.2% (24/52) had the highest distribution while *Proteus* spp. 11.5% (6/52) had the least distribution. Ciprofloxacin was the most sensitivity (100%) while Amoxicillin was the most resistant (38%). There was a high prevalence of asymptomatic bacteriuria in this study. The most commonly observed organisms were *Escherichia coli*. Ciprofloxacin was the most sensitive antibiotics and there was a widespread antibiotic resistance in this study. It is therefore recommended that screening among diabetic patients for urinary tract infections, sensitization and strategies to promote effective drug usage be encouraged.

Keywords

Antibiogram, Asymptomatic Bacteriuria, Diabetes, Uropathogens.

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INTRODUCTION

Diabetes is a group of frequently occurring metabolic disorders which has similar physical properties with hyperglycemia. It is associated with decreased insulin synthesis and utilization, which impairs the body's ability to ineffectively use nutrients (1). Urinary tract infections which are usually classified as symptomatic and asymptomatic are reported to be common infections among diabetic patients (2).

Diabetics are more likely to get urinary tract infections because their immune systems are weaker and they often have problems with their bladder control, which makes them more likely to let bacteria and uropathogens get into their genitals. The high level of glucose in their urine makes it perfect for these organisms to grow (3,4). There is a significant increase in urinary tract infections in people with diabetes, which is likely caused by the presence of other infections (uropathogens in the kidneys) (5).

Most pathogens that can cause urinary tract infections in people with diabetes are very likely to change their antibiotic resistance patterns over time. This means that many of these pathogens are less likely to respond to antibiotics (6-8). O'Neil et al., (9) has reported that a rise in antibiotics resistance could result to global mortality of ten million annually. Due to the health importance of the pathogens which cause urinary tract infections, it is germane to adequately identify the causative agents and the effective antimicrobial agents against them for successful treatment of the infections (10). Despite the fact that there have been several researches on antibiotic resistance, the situation still exists and requires pragmatic investigations that will provide up-to-date information on antimicrobial resistance. There is also paucity of data on the asymptomatic bacteriuria in patients with diabetes in Calabar. This study was to identify the pathogens associated with asymptomatic bacteriuria among patients with diabetes and the antibiogram of those isolates in Calabar. This will provide information on the asymptomatic bacteriuria in diabetic patients and provide guide for treatments.

MATERIALS AND METHODS

Study area

The area of study is Calabar, the capital of Cross River State, which includes the Calabar Municipal and Calabar South Local Government Areas in the South-South region of Nigeria.

Study design

This was a cross-sectional study of 200 healthy asymptomatic diabetic patients in Calabar who volunteered to take part in the study. Personal information was obtained from each of the study participants. The inclusion criterion was participants from the ages of 21 years with history of diabetes while the exclusion were participants with participants with pregnancy, signs and symptoms of urinary tract infections, antibiotic usage within one week preceding the study and history of underlying illness and less than 21 years.

Specimen collection and processing

Early morning midstream urine and blood specimens were aseptically obtained from each diabetic patient, labeled and transported to the laboratory for processing. The glucometer described by Pickering & Marsde (11) has been used to measure the level of fasting blood sugar in the blood.

Macroscopy of the urine specimens was carried out in the laboratory to examine for the physical properties of the urine samples. The samples were analyzed microscopically by centrifuging 10 mL of each sample in a test tube at 3,000 revolution per minute (rpm) for 5 minutes. The supernatant was poured off while the sediment was well mixed for a wet preparation and a drop of the sediment was placed on a clean, grease-free glass slide and covered with cover glass. The preparation microscopically examined using the 10X and 40X objectives with the condenser iris closed sufficiently to provide good contrast of white blood cells (leukocytes), red blood cells (erythrocytes), bacterial debris and casts (12).

The sample was inoculated with 1μL of a standard quantitative loop to a cysteine lactose electrolyte deficient (CLED) agar, MacConkey, and Blood agar plates (Oxoid, Ltd., Basingstoke, Hampshire, England). The plates were incubated aerobically at 37^{o}C for 24 hours. The result was reported as significant/non-significant growth, or contaminated (discarded). Urine culture plates showing ≥105 colony-forming units (CFU)/mL of single bacterial species were considered as significant bacteriuria (13).

The presumptive identification criteria of the organisms were Gram-stain reaction of the organisms, microscopic appearance and colony characteristics. Indole production, citrate utilization, H_2S production, gas production, urea hydrolysis, lysine decarboxylation, lactose fermentation and motility were used for further identification of Gram-negative bacteria. Coagulase, catalase, and mannitol fermentation assays were used to further identify Gram-positive bacteria (12).

Antibiogram (antiobiotic testing) test was performed on all positive isolates using the standardized Kirby Bauer disc diffusion technique according to the criteria of the Clinical and Laboratory Standards Institute (14). Antibiotic-impregnated discs containing Amoxicillin-Clavulanic acid (AMC, 30μg), Pefloxacin (DEF, 30μg), Gentamycin (GN, 10μg), Chloramphenicol (CH, 10μg), Ciprofloxacin (CIP, 5μg), Sulfamethoxazole/Trimethoprim (SXT, 30μg), Augmentin (AU, 30μg) and Septrin

(SP, 30µg) were placed onto the surface of Mueller-Hinton agar.

Statistical analysis

Data obtained were analyzed using the Statistical Package for Social Science (SPSS) version 20 manufactured by International Business Machines (IBM Corp, Armonk, New York). Proportions were used for categorical variables. Differences in infection rates among participants were determined by Chi-square and P-value <0.05 was considered significant.

Ethical approval

This was sought for and obtained from the Cross-River State Health Research Ethics Committee of the Cross-River State Ministry of Health Research Ethics Committee (CRS-HREC) with approval number: CRS/MH/HREC/020/Vol.V1/255 and a written consent form was also duly signed by the participants before taking part in the study.

RESULTS

The mean fasting blood sugar levels of the study cohort by age is shown in Table 1. The highest mean fasting blood sugar level was among those between the ages of 41-50 years (12.3±4.38) while the least mean fasting blood level was among those between the ages of 21-30 years (8.9±2.84).

Table 1. Mean Fasting Blood Sugar of the Study Population by Age

Age (years)	No. examined	Mean Fasting blood sugar (FBS) level (mmol/L)
21-30	12	8.9±2.84
31-40	48	11.2±4.65
41-50	48	12.3±4.38
51-60	58	10.6±3.59
61-70	34	11.0±3.77
Total	200	11.08±4.07

The mean fasting blood sugar of the study population by gender as presented in Table 2 shows that the fasting blood level of the males (11.3±4.46) was higher than that of the females (10.9±3.70).

Table 2. Mean Fasting Blood Sugar of the Study Population by Gender

Gender	No. examined	Mean FBS level
Male	98	11.3±4.46
Female	102	10.9±3.70
Total	200	11.08±4.07

The prevalence of bacterial pathogens in the study is 26% based on the incidence of infections among participants by age (Table 3). The highest rate of infection was found in those between the ages of 41 and 50 (37.5%) while the lowest rate was found in people between the ages of 61 and 70 (11.8%). The difference between the rate of infection and age was not statistically significant (*P*>0.05).

$$\text{Prevalence of bacterial pathogen} = \frac{\text{Total number of infected participants}}{\text{Total number of the study population}} \times 100$$

Table 3. Occurrence of Infections of the Study Population by Age

Age (years)	No. examined	No. (%) infected (n=52)	Statistics
21-30	12	2 (16.7)	$\chi2$=37.5245, *P*=0.1106 at df=4
31-40	48	12 (25.0)	
41-50	48	18 (37.5)	
51-60	58	16 (27.6)	
61-70	34	4 (11.8)	
Total	200	52 (26.0)	

According to Table 4, females had a greater infection rate of 29.4% (30/102) than males did (22.4%, 22/98). Gender differences in infection rates were not statistically significant (P>0.05). Table 5 lists the biochemical processes used to determine the presence of bacterial pathogens.

Table 4. Occurrence of Infections in the Study Population by Gender

Age (years)	No. examined	No. (%) infected (n=52)	Statistics
Male	98	22 (22.4)	$\chi2$=0.1380, *P*=0.7102 at df=1
Female	102	30 (29.4)	
Total	200	52 (26.0)	

Table 5. Biochemical Reactions of Pathogenic Bacteria in Urine Samples

Biochemical reaction	Bacterial pathogen			
	E. coli	***Klebsiella***	***Staphylococcus aureus***	***Proteus***
Gram reaction	-ve	-ve	+ve	-ve
Microscopic appearance	Rod	Rod	Spherical	Rod
Colony on CLED agar	Opaque yellow	Yellow mucoid	Golden yellow	Translucent blue
Colony on MacConkey agar	Pink	Pink	Red-pink	Colourless
Colony on blood agar	Yellow	Grey-white mucoid	Yellow	Grey-white swarm
Indole	+ve	-ve	-ve	-ve
Citrate	-ve	+ve	+ve	+ve
Urease	-ve	+ve	+ve	+ve
Lysine	+ve	-ve	+ve	-ve
Lactose	+ve	+ve	+ve	-ve
H_2S	-ve	-ve	-ve	+ve
Gas production	+ve	+ve	-ve	+ve
Motility test	+ve	-ve	-ve	+ve
Coagulase			+ve	
Catalase			+ve	
Mannitol			+ve	

Key: +ve = positive, -ve = negative

Bacterial pathogens in the study population showed that *Escherichia coli* had the highest prevalence at 46.2% (24/52), as show in Table 6. Conversly, *Proteus* spp. had the lowest distribution of 11.5% (6/52).

Table 6. Occurrence of Bacterial Pathogens in the Study Population (n=52)

Bacteria	Frequency of occurrence (%)
Escherichia coli	24 (46.2)
Klebsiella spp.	14 (26.9)
Staphylococcus aureus	8 (15.4)
Proteus spp	6 (11.5)
Total	**52 (26.0)**

Table 7 shows the rate of growth inhibition to bacterial pathogens by antibiotics. Ciprofloxacin was fully susceptible (100%) to all the bacterial pathogens. Gentamicin is primarily sensitive to *E. coli* (87.5%), Pefloxacin was mostly sensitive to *Klebsiella* (71.4%), Septrin was mostly sensitive to *Klebsiella* and *Proteus* (50%), Augmentin was mostly sensitive to *Staphylococcus aureus* (50%), Chloramphenicol and Amoxillin were mostly sensitive to *E. coli* (45.8% and 41.7% respectively). Ciprofloxacin (CPX) was the most sensitivity (100%) on the isolates while Amoxicillin (AM) was the least sensitivity (46.2%) as presented in Figure 1.

DISCUSSION

Urinary tract infections are more frequent with more severity among those with diabetes which are most times caused drug resistant microorganisms (15). In this study, people with diabetes had a significant prevalence (26.0%) of urinary tract infections. This was lower than the prevalence of urinary tract infections among the same subjects as was previously reported by Shah et al., (16) in Malaysia, Dave et al., (17) at Ahmedabad in India, 92.0% and higher than 10.6% by Worku et al., (18) at Debre Tabor and 10.7% by Mohammed et al., (19) at Hawassa both in Ethiopia. These disparities in prevalence might have been as a result of the variations in sample size, geographical location, personal hygiene, and the screening tests used.

The highest risk of infection was found in people between the ages of 41 and 50. This was consistent with the findings of other studies that majority of asymptomatic bacteriuria occurred among adults over the age of 40 (17,20). This may be due to physiologic changes related to aging and comorbid illnesses in elderly adults. Females were mostly infected in this study. Similar reports have been made by Nabaigwa et al.,

(21) Kumar et al., (22) and Nadia et al., (23) who noted that females were more higher infections in than males. Females are susceptible to bacteriuria due to the shorter distance between the female urethra and the anus compared to the male urethra.

Table 7. Inhibition Rate of to Pathogenic Bacteria by Antibiotics

Bacterial pathogen	Sensitivity to antibiotics (%)						
	Cipro	Gen	Pef	Sep	Aug	Chlo	Amo
E. coli (n=24)	24 (100)	21 (87.5)	17 (70.8)	10 (41.6)	9 (37.5)	11 (45.8)	10 (41.7)
Klebsiella spp (n=14)	14 (100)	12 (85.7)	10 (71.4)	7 (50.0)	5 (35.7)	6 (42.9)	5 (35.7)
S. aureus (n=8)	8 (100)	5 (62.5)	5 (62.5)	3 (37.5)	4 (50.0)	3 (37.5)	3 (37.5)
Proteus spp (n=6)	6 (100)	4 (66.7)	3 (50.0)	3 (50.0)	2 (33.3)	1 (16.7)	2 (33.3)

Key: Cipro=Ciprofloxacin, Gen=Gentamicin, Pef=Pefloxacin, Sep=Septrin, Aug=Augmentin, Chlo=Chloramphenicol, Amo=Amoxicillin

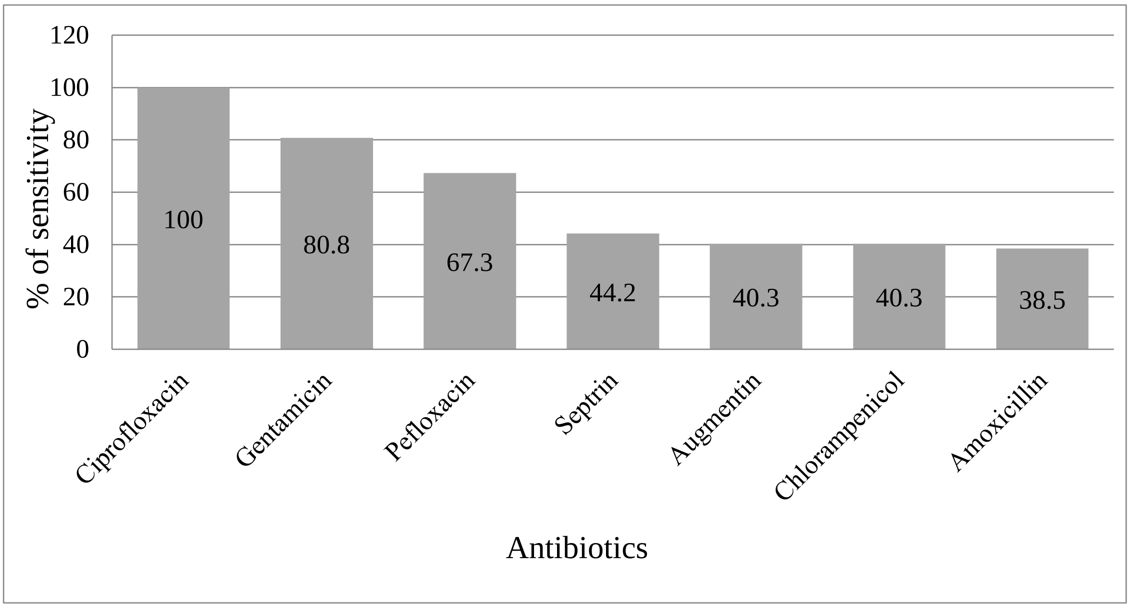

Figure 1. Percentage of Sensitivity of Antibiotics

Escherichia coli were the most observed organisms (46.2%) in this study followed by *Klebsiella* spp. (26.9%). The findings of Akram et al., (24), Kalaichelvi & Daranendaranchellapa (25), Durmaz et al., (26) and Bhagat & Sahu (27), who observed that *Escherichia coli* were the most uropathogens concurred with this. This might have happened as a result of *Escherichia* a typical human intestine bacterium that can quickly turn opportunistic in the urinary tract. Most of the isolates were resistant to the tested antibiotics in this study. This high antibiotic resistance is a global issue which is commonly due to abuse of antibiotics (28). The study area reported that antibiotics were severly misused and significantly associated with antibiotic resistance (29).

CONCLUSIONS

The prevalence of asymptomatic bacteriuria among diabetic patients in Calabar was 26.0%. The most commonly observed organisms were *Escherichia coli*. The study found that widespread antibiotic resistance existed and that ciprofloxacin was the most sensitive medication. In order to prevent complications, it is advised that diabetes individuals regularly get checked for urinary tract infections. A study with larger sample size and power should be conducted to evaluate the distribution of uropathogens among diabetic patients. Patients should be educated about the appropriate antibiotic use based on culture results. Implementation of management program to explain the usage of antibiotic is needed.

AUTHOR CONTRIBUTIONS

Etefia U. Etefia and Sonia O. Ejiofor: designed the study. Etefia U. Etefia: analyzed the study data. Paul C. Inyang-Etoh: approved the final version for submission. All authors critically reviewed the manuscript.

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CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

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