Frequency and antibiotic susceptibility pattern of uropathogenic agents of urinary tract infections among asymptomatic diabetic patients in Okada community, southern Nigeria

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Summary

Massive evidence showed that patients with diabetes have a high risk of urinary tract infections. We studied the frequency of potential urinary uropathogens among diabetic patients and identified their antimicrobial susceptibility patterns.

This was a prospective hospital-based study conducted at the Department of Medical Laboratory Science, Igbinedion University Teaching Hospital, Southern Nigeria, between January 2014 and May, 2014. We included 240 previously confirmed diabetic patients (women, n=70 and men, n=170) who were regularly followed up without prior treatment with any antimicrobial therapy and within the age range of 26-75 years. Patient personal history data and midstream urine samples were collected. Urine samples were processed in the laboratory following a Standard Laboratory Protocol.

Escherichia coli, Staphylococcus aureus, *Klebsiella* spp. and *Candida* spp. were isolated in this study. A significant bacteriuria count was estimated in 12.5% of the sampled population, while 17.1 and 10.6% were estimated in females and males, respectively. Similarly, candiduria was found in female, male and total sample in 12.9%, 2.9%, and 5.8%, in that order. According to antimicrobial sensitivity testing, the Gram-negative bacilli isolated were highly sensitive to nitrofurantoin followed by ofloxacin, gentamycin and least sensitive to cefuroxime. Estimation of potential uropathogens among asymptomatic diabetic populations may avert possible urinary tract infections and their possible complications ultimately and thus prevent possible advanced renal diseases.

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Introduction

Diabetes mellitus alters the genitourinary system where urinary tract infection (UTI) could prompt urological problems, such as dysuria, organ damage and sometimes even death due to complicated UTIs involving pyelonephritis (21), emphysematous cystitis, renal abscesses and renal papillary necrosis (16).

UTI has been repeatedly reported in diabetic patients (3,8,20,22). Several mechanisms unique to diabetes have been implicated as potential factors that may increase the risk of developing UTI (3). Some of these factors involve increase in urine glucose level that may possibly enrich growth of pathogenic bacteria (8). Several reports noted that high renal parenchymal glucose level supports a conducive milieu towards multiplication of these uropathogens, thus indicating a driving factor for developing pyelonephritis and other renal complications − emphysematous pyelonephritis and renal abscesses (20,22). Furthermore, various impairments to the immune system affecting cellular, innate and humoral immunity may contribute to the development of UTIs in diabetics (5,9).

The frequency of UTI in diabetic patients has been assessed to vary from 8% to 26% in different regions (8,10,28). Estimating frequency of potential uropathogens along with their antimicrobial drug susceptibility may help improve the management of diabetic patients who are most times asymptomatic and thus help prevent UTIs and their complications.

Materials and Methods

Study area

This study was carried out at Igbinedion University Teaching Hospital (IUTH) Okada, Edo State, Southern Nigeria after obtaining ethical approval. All participants gave informed consent.

Study population

The sample comprised male (n=170) and female (n=70) patients attending the diabetes clinic; their age ranged from 26 to 75 yrs. Detailed information of the patients regarding age, sex and plasma glucose level at time sampling was obtained during the exercise.

Selection criteria

Inclusion criteria

Diagnosis of diabetes was based on glucose values (fasting plasma glucose of ≥126 mg/dL; 2-hour post prandial ≥200 mg/dL) according to WHO criteria (27).

Exclusion criteria

We excluded patients on antibiotic therapy and pregnant women who have been reported in previous studies with possible increase in incidence of UTIs, owing to pregnancy-associated urinary stasis and vesicoureteral reflux (13,24).

Specimen collection

Clean voided midstream urine samples were collected into sterile universal containers with identification number, sex and age well written on the bottles after giving proper instructions. Every individual patient submitted one specimen each. The samples were then transported to the laboratory immediately and for processing within 30 to 60 minutes of voiding.

Isolation of microorganisms

Each urine sample was properly mixed before streaking on agar plates with the aid of a sterile wire loop. The urine samples were inoculated on dried plates of MacConkey agar, cysteine lactose electrolyte deficient (CLED) agar, mannitol salt agar, and Sabouraud Dextrose agar (Oxoid, UK). The plates were incubated aerobically at 37°C for 24-48 hrs and were observed for growth after incubation. The isolates were considered with the colony count >10^5 CFU/mL (1).

Characterization and identification of isolated microorganisms

Colony identification and confirmation were done for the bacterial isolates using standard microbiological techniques including Gram staining reactions, colony growth morphology on media, lactose fermentation, catalase, coagulase, oxidase, indole, citrate utilization and urease tests. Fungal isolates on the Sabouraud agar plates were characterized and identified as *Candida* spp. by colony morphology and Gram staining, while phenotypic identification was furthered using germ tube test (GTT) for speciation of the yeast (12).

Antimicrobial sensitivity testing

Antibiotic susceptibility of the isolates was carried out on sterile Mueller Hinton agar using the Kirby Bauer disc diffusion method (1). The agar surface was evenly inoculated using a sterile swab loaded with a cell suspension adjusted to the turbidity of 0.5 McFarland standards (12). The following antibacterial disks were used: ceftazidine (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), amoxicillin-clavulanic acid (30 µg), nitrofurantion (300 µg), ampicillin (10 µg), erythromycin (15 µg) and ceftriaxone (30 µg) (BioRad) following the manufacturer instructions. The plates were left out on the bench for 30 mins before incubation.

Measurement of inhibition zones

After 24 hours of incubation at 37°C, the cultured plates were examined and the diameter of inhibitory zones was measured. Susceptibility or resistance was determined according to the CLSI guidelines and published papers (7,12,23).

Statistical

The results obtained in this study were analysed using descriptive statistics: proportion, frequency and percentage to assess the level of variability.

Results

The cases with positive cultures of bacterial isolates were 12 (17.1%) females and 18 (10.6%) males; overall, the cases of bacteriuria were 30 (12.5%).

Similarly, candiduria was found in 14 (5.8%) of the whole sample: 9 (12.9%) in females, and 5 (2.9%) in males (Table 1).

Different age groups showed varying proportions of positive cases of uropathogens among the diabetics. Highest frequencies (46.7% cases) of asymptomatic bacteriuria were found among the age group of 36-45 years, followed by 40% cases in 46-55 yrs group. Other age groups (26-35 and 55-65 years) equally had 6.6% cases each, respectively (Table 2).

Frequency of total significant uropathogens in diabetic patients shows that 22 (50.0%) were Gram negative bacilli (GNB) constituting 16 (36.4%) *Escherichia coli* and 6 (13.6%) *Klebsiella* spp. Other bacterial isolates were Gram positive cocci (GPC) consisting only *Staphylococcus aureus* (n=8; 18.2%), while the remaining significant isolates were 14 (31.8%) *Candida* spp. (Figure 1).

Also, with antibiotics susceptibility testing, GNB was found most sensitive to nitrofurantoin followed by ofloxacin, gentamycin and least sensitive to cefuroxime (Table 3).

Discussion

Urinary tract infection (UTI) is a possible clinical problem in people suffering from diabetes mellitus. Many studies previously reported prevalence of asymptomatic bacteriuria (ASB) in diabetic population as high as 8-26% (10,22,28). This is in agreement with the present study. We found frequency of 12.5% of diabetic patients in our sample population as ASB, while 17.1 and 10.6% indicated the percentage frequencies for female and male subgroups, respectively.

Importantly, women were identified with more significant frequencies of uropathogenic agents in this study and this was in concordance with previous studies (2,14,15). This may be due to a short urethra length which is in proximity to the moist and warm vulvar region and perianal area or attributed to higher level of recurrence of UTI in women (11). Generally, these areas are often colonized by enteric bacteria (14,15). This anatomical position often allows bacteria to travel up the urinary tract length and thus become uropathogenic in this new environment (4,18). Generally, we presumed that increase in glucose concentrations of diabetic's urinary flow along the tract may possibly play a contributory role in this case and could set off UTI in diabetics if left uncontrolled (26).

In addition, the results of our study indicated that increase in age may not influence a chance or occurrence of UTI. Likewise, it was previously reported that age was not associated with complicated course of UTIs in patients with diabetes thus not a useful predictor (11). Although, UTIs were not noticed during patients' recruitment in our study. On the other hand, there are different reports in contrast to our findings. Some studies show that ASB increases with age, and is also associated with urinary tract abnormalities (4,18).

Further, the largest number of uropathogens isolated in the present study was Gram negative bacilli (GNB). This corroborates a bacteriological study where Gram negative enteric organisms, such as *E. coli, Klebsiella* spp. and *Proteus* spp., are implicated with ASB (26). Likewise, the pattern of isolation of organism in our study was similar to a previous result involving various regions implicating GNB, particularly *E. coli*, as the commonest pathogen of both ASB and UTIs (19,26). Also, our study is in agreement with a report from India population, where *E. coli* is the most frequently reported (64.3%) uropathogens, followed by *S. aureus* (21.4%) and *Klebsiella* spp. (14.3%) (6). In tandem with the current study, similar frequency of *E. coli* was predominantly isolated, followed by *S. aureus* before *Klebsiella* spp. Although it was very infrequent, we found some candida growths during post-culture characterization and identification of the organisms. This is in agreement with previous finding where type 2 diabetes is reported as a risk factor for fungal UTI, attributed to *Candida* spp. as the commonest fungal agent (25).

Furthermore, our findings on antimicrobial susceptibility testing showed that nitrofurantoin was the most sensitive drug followed by ofloxacin and gentamycin, against the isolated uropathogens. This is in contrary to some reports where quinolones were found most effective against isolated GNB (28). This may be attributable to changing trends in antimicrobial susceptibility pattern from place to place, with possibility of diverse genetic variations. Moreover, our results showed that the isolated organisms were resistant to majority of the first line antibiotics and may thereby require the need of newer and more effective antibiotics for safe treatment.

Conclusions

Findings from this study showed that significant asymptomatic bacteriuria accounted for frequency of 12.5% in total dia-

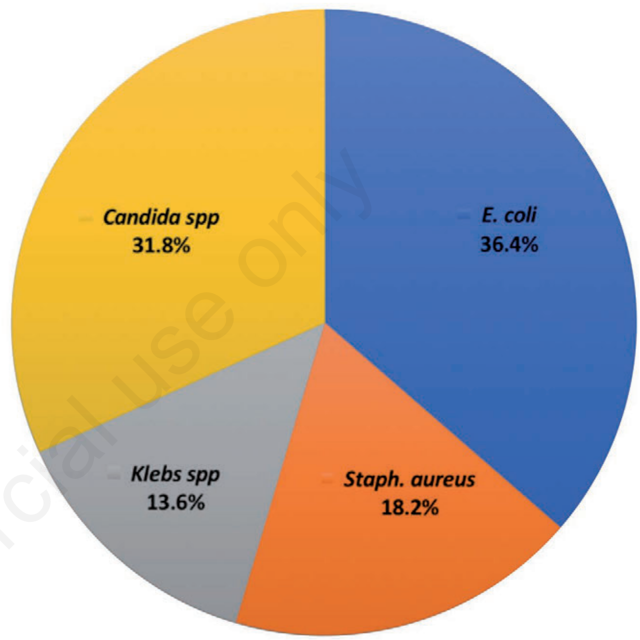

Figure 1. Frequency of isolates in asymptomatic diabetic patients.

Table 1. Gender distribution in diabetic patients.

Uropathogens	Gender	Frequency (%)	No. significant positive culture cases	No. cases examined
Bacterial isolates	F	17.1	12	70
	M	10.6	18	170
	Total	12.5	30	240
Candida spp.	F	12.9	9	70
	M	2.9	5	170
	Total	5.8	14	240

Table 2. Age distribution of asymptomatic bacteriuria in diabetic patients.

Age group	Male, %	Female, %	Total population, n. (%)
26-35 years	2	0	2 (6.6)
36-45 years	10	4	14 (46.7)
46-55 years	6	6	12 (40)
55-65 years	0	2	2 (6.6)
Total	18	12	30 (100)

Table 3. Antimicrobial sensitivity/resistance pattern of bacterial isolates in asymptomatic diabetic patients.

Antimicrobials, sensitive	*Escherichia coli* Male (n=10)	*Escherichia coli* Female (n=6)	*Klebsiella* spp. Male (n=2)	*Klebsiella* spp. Female (n=4)	*Staphylococcus aureus* Male (n=6)	*Staphylococcus aureus* Female (n=2)
Ceftazidine	0/10	0/6	2/2	2/4	0/6	0/2
Cefuroxime	0/10	0/6	2/2	0/4	0/6	0/2
Gentamicine	6/10	4/6	0/2	2/4	4/6	2/2
Ciprofloxacin	4/10	6/6	0/2	2/4	-	-
Ofloxacin	6/10	6/6	0/2	2/4	4/6	2/2
Augmentin	4/10	0/6	0/2	0/4	2/6	0/2
Nitrofurantion	10/10	6/6	0/2	4/4	-	-
Ampicillin	0/10	0/6	0/2	0/4	-	-
Erythromycin	-	-	-	-	6/6	2/2
Ceftriaxone	-	-	-	-	0/6	0/2

betic population, while 17.1 and 10.6% were estimated in females and males respectively. Significant numbers of positive isolates of *Candida* spp. in female, male and sampled populations were 12.9%, 2.9%, and 5.8%, in that order. We found that *E. coli* and *Candida* spp. were the most frequent potential uropathogens in diabetic patients, even though the patients were asymptomatic. Consequently, investigation or screening for these critical organisms could be advisable for improved management and prevention of full blown UTI and its potential complications or advanced renal problems. For this reason, assessment of potential significant uropathogens is recommended for diabetic population.

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