Observed changes in the prevalence of uropathogens in Benin City, Nigeria

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Abstract
Objective: Against the background of reports of changes in the prevalence of uropathogens, this study aimed to determine the prevalence of asymptomatic bacteriuria among out-patients of a tertiary hospital, the most prevalent uropathogen, and the distribution of uropathogens among both genders.
Methods: Clean–catch midstream urines were collected from 1,238 out–patients consisting of 1033 non–diabetic patients (451 males and 582 females) and 205 diabetic patients (93 male and 112 females). The diabetic patients consisted of 66 type 1 and 139 type 2 diabetes. Significant bacterial isolates were identified in the urine samples using standard techniques.
Results: There was a significant difference ($p < 0.001$) in the prevalence of asymptomatic bacteriuria between non–ddiabetic and diabetic patients (24.5% vs 59.0%). Type of diabetes did not affect the prevalence of asymptomatic bacteriuria (type 1: 53.03%; type 2: 61.87%; $p > 0.05$). Female non–diabetic patients had significantly higher prevalence of asymptomatic bacteriuria ($p < 0.001$). *Staphylococcus aureus* was the most common uropathogen (26.03%) as well as in both genders of diabetic and non–diabetic patients.
Conclusions: An overall prevalence of 30.29% of asymptomatic bacteriuria was found and *Staphylococcus aureus* was the predominant uropathogen in both genders of out–patients.

Keywords: Asymptomatic bacteriuria, prevalence, uropathogens, diabetes, *Staphylococcus aureus*

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Introduction
Urinary tract infections (UTI) are among the most common conditions causing individuals to seek medical care (1). They are also among the most common bacterial infections in humans, both in the community and hospital settings, occur in all age groups, in both genders, and usually require urgent treatment (2). Urine is the most received and processed specimen in a clinical microbiology laboratory (3) and *Escherichia coli* has been reported as the most prevalent aetiological agent (1,2,4,5). However, two separate studies – one in Ibadan, Nigeria (6) and the other in Kampala, Uganda (7), in 1969, reported *Staphylococcus aureus* as the predominant isolate causing UTI among pregnant women. In 2003, *Pseudomonas aeruginosa* was reported as the predominant isolate causing asymptomatic UTI among residents of Zaria, Nigeria (8) and in 2006, *Staphylococcus aureus* was the most prevalent isolate causing asymptomatic UTI among pregnant women in Ibadan, Nigeria (9). Against this background, this study aimed to determine the prevalence of asymptomatic bacteriuria (AB) among out–patients attending various clinics in a tertiary hospital in Benin City, Nigeria, to determine the most prevalent uropathogen, and the distribution of uropathogens among both genders.

Materials and methods
Study population
The study was carried out at the University of Benin Teaching Hospital, Benin City, Nigeria for a period of six months. A total of 1,238 patients were studied. They comprised 1033 non–diabetic (non–DM) patients (451 males and 582 females) and 205 diabetes mellitus (DM) patients (93 male and 112 females). The DM patients consisted of 66 type 1 DM (30 males and 36 females) and 139 type 2 DM (63 males and 76 females) patients. The study subjects were out–patients attending various clinics. Exclusion criteria included signs and symptoms of UTI, antibiotic usage within one week and large fluid in–take prior (less than one hour) before clinic attendance. Verbal informed consent was obtained from all patients prior to specimen collection. Approval for the study was given by the Ethical Committee of the University of Benin Teaching Hospital.

Specimen collection and processing
Clean–catch midstream urine was collected from each patient into a sterile screw–capped universal container, containing a few crystals of boric acid as preservative. The specimens were mixed, labeled and transported to the laboratory for processing.

A loop-full (0.001mL) of well mixed un-centrifuged urine was streaked onto the surface of blood agar and cystine lactose electrolyte deficient (CLED) medium (M6: Plasmatec Laboratories, United Kingdom). The plates were incubated aerobically at 370C for 24 hours and counts were expressed in colony forming units (CFU) per millilitre (mL). A count of $\geq$ 105 CFU/mL was considered significant to indicate asymptomatic bacteriuria.

Ten mL of each well-mixed urine sample was centrifuged at 2000 g for 5 minutes. The supernatant was discarded and a drop of the deposit was examined microscopically at high magnification for pus cells, red blood cells, epithelial cells, casts, crystals yeast-like cells and *Trichomonas vaginalis*. Pus cells $\geq$ 5 per high power field were considered significant to indicate infection. The isolates were identified by standard microbiological methods (10).

Statistical analysis was by the Chi (X2) square test . A p value of <0.05 was deemed statistically significant.

Results
There was a significant difference in the prevalence of AB between non–DM and DM patients (24.59% vs 59.02% respectively; $p<0.001$). The prevalence of AB did not differ significantly between type 1 DM and type 2 DM patients (Table 1). Females showed a higher prevalence of AB than males, but only significant in the non-DM patients (Table 1).

A total of 484 microbial isolates were recovered and *Staphylococcus* aureus was the most predominant isolate irrespective of patient type (Table 2). Table 3 shows the distribution of uropathogens among gender. With the exception of females from non–DM and type 1 DM patients, *Staphylococcus aureus* was the predominate isolate in both genders. In females from non–DM patients, *Candida albicans* was the most prevalent isolate (29.65%) while in females with type 1 DM, both *Candida albicans* and *Staphylococcus aureus* predominated with a frequency of 30% each.

Table 1. Distribution of asymptomatic bacteriuria among gender

Subjects	Gender					
	Male		Female (%)		Total	
	n	n infected (%)	n tested	n infected (%)	n	n infected (%)
Non–DM†	451	75 (16.63)	582	179 (30.76)‡	1033	254 924.590
Type 1 DM	30	12 (40.00)	36	23(63.89)	66	35(53.03)
Type 2 DM	63	35 (55.56)	76	51 (67.11)	139	86 (61.87)
Total	544	122 (22.43)	694	253 (36.46)	1238	375(30.29)
† Non–DM vs DM : p < 0.001. ‡ Male vs Female : p < 0.001						

Table 2. Prevalence of uropathogens.

Organism	Non–DM patients (%)	DM patients		Total (%)
		Type 1 (%)	Type 2 (%)	
Escherichia coli	65 (21.81)	9 (20.00)	29 (20.57)	103 (21.28)
Klebsiella species	16 (5.37)	4 (8.89)	15 (10.64)	35 (7.32)
Proteus species	31 (10.40)	1 (2.22)	11 (7.80)	43 (8.88)
Pseudomonas aeruginosa	16 (5.37)	1 (2.22)	8 (5.67)	25 (5.17)
Staphylococcus aureus	76 (25.50)	13 (28.89)	37 (26.24)	126 (26.03)
Coagulase negative Staphylococci	22 (7.38)	2 (4.44)	10 (7.09)	34 (7.02)
Enterococcus faecalis	1 (0.34)	5 (11.11)	9 (6.38)	15 (3.10)
Candida albicans	71 (23.83)	10 (22.22)	22 (15.60)	103 (21.28)
Total	298 (61.57)	45 (9.30)	141 (29.13)	484 (100)

Table 3. Distribution of uropathogens among gender.

Organism	Non DM		DM patients			
			Type 1		Type 2	
	Male (%)	Female (%)	Male (%)	Female (%)	Male (%)	Female (%)
Escherichia coli	21(21.21)	44 (22.11)	3 (20.00)	6 (20.00)	10 (18.87)	19 (21.59)
Klebsiella species	6 (6.06)	10 (5.03)	2 (13.33)	2 (6.67)	6 (11.32)	9 (10.23)
Proteus species	14 (14.14)	17 (8.54)	1 (6.67)	0 (0.00)	6 (11.32)	5 (5.68)
Pseudomonas aeruginosa	10 (10.10)	6 (3.02)	1 (6.67)	0 (0.00)	5 (9.43)	3 (3.41)
Staphylococcus aureus	31 (31.31)	45 (22.61)	4 (26.67)	9 (30.00)	11 (20.75)	626 (29.55)
Coagulase negative Staphylococci	4 (4.04)	18 (9.05)	1 6.67)	1 (3.33)	4 (7.55)	6 (6.82)
Enterococcus faecalis	1 (1.01)	0 (0.00)	2 (13.33)	3 (10.00)	4 (7.55)	5 (5.68)
Candida albicans	12 (12.12)	59 (29.65)	1 (6.67)	9 (30.00)	7 (13.21)	15 (17.08)

Discussion

Several reports exist, indicating changes in the prevalence of uropathogens (6-9). Against this background, this study focused on determining the prevalence of AB among out-patients (diabetic and non–diabetic) of a tertiary hospital as well as to determine the most prevalent uropathogen and the distribution of uropathogens among genders of the study population.

Our study showed a prevalence of AB of 30.29%. Non–DM patients had a significantly lower prevalence than DM patients and this agrees with earlier reports (4,11). High glucose concentration in urine which may create a culture medium for pathogenic microorganism and immunologic impairment resulting in lower host defense system, have been reported to increase the risk of AB in diabetics (4).

DM patients on insulin therapy have been reported to have a higher prevalence of AB (4,11). Severity of diabetes, which may be indicated by insulin use (4), is a possible reason for this difference. However, in this study, type 2 DM patients had a higher prevalence of AB than type 1 DM patients, though the difference failed to reach statistical significance.

The finding that females had higher prevalence of AB than males agrees with earlier studies (1,12). However, in this study, statistical significance was observed among non–DM patients only ($p < 0.001$). Close proximity of the female urethral meatus to the anus, shorter urethra, and sexual intercourse have all been reported as factors that influence the higher prevalence in females (1,2).

The aetiological agents associated with AB are similar in both DM and non–DM patients. A total of 484 isolates were recovered from 375 specimens with AB, indicating mixed infections in some patients. *Staphylococcus aureus* was generally, the most common isolate and in both DM and non–DM patients. Also, *Staphylococcus aureus* was the most prevalent isolate in both genders, with the exception of female non–DM patients, where it was second in prevalence to *Candida albicans. Staphylococcus aureus* is a normal flora of female perineum and vulva (13) and during sexual intercourse can easily be carried into the urethra by a massaging process. Also, staphylococci are part of vagina flora (13) and manipulations that alter vaginal flora, such as insertion of a contraceptive device – a known risk factor for UTI (14), can result in opportunistic UTI infection with this organism.

The reason for the high prevalence of *Staphylococcus aureus* in males is not clear, though lack of circumcision, receptive anal intercourse and HIV infection are recognized risk factors for UTI in males (2).

We also noted that these observed changes in the prevalence of uropathogens are mostly form Africa as *Escherichia coli* remains the most common aetiological agent in North America (15). This may indicate that these changes occur in some geographical locations. The changes may also be transient as they were first reported in 1969 (6,7) and later in 2003 and 2006 (8,9). However, these will require further investigations to verify.

The observed changes in this study have serious implications as most clinicians treat patients without recourse to laboratory guidance (2). Such treatments are usually based on known aetiological agents and susceptibilities. This observed change in the prevalence of uropathogens may lead to change in antimicrobial susceptibility and ineffective treatment. Therefore, clinicians should rely on laboratory guidance before therapy as this will overcome the problem of mistreatment and reduce the emergence of resistant uropathogens.

In conclusion, our study revealed a prevalence of 30.29% of AB among out–patients of University of Benin Teaching Hospital, Benin City. The prevalence was higher in DM patients and *Staphylococcus aureus* was the most prevalent uropathogen in both genders of DM and non–DM patients. Further studies are needed to ascertain if this change in the prevalence of uropathogens are transient and restricted to certain geographical locations.

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