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Pattern of microbial flora and antibiotic sensitivity among healthy adults with bacteriuria in a university community in south-eastern Nigeria

VITALIS OBISIKE OFURU [1, *], JENNIFER ONUZULIKE [2] and OKIGBEYE DANAGOGO [1]

[1] *Department of Surgery, Urology Division, University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State, Nigeria.*
[2] *Department of Health Services, Federal University of Technology, Owerri (FUTO), Imo State, Nigeria.*

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Abstract

Background: Knowledge of common urine microbial flora and the sensitivity pattern are essential in determination of empirical antibiotic usage

Aim: To determine the common microbiological flora in the urine of apparently healthy adults and the sensitivity and resistance pattern.

Materials and Methods: A cross sectional survey of the pattern of urinary microbial organisms and their sensitivity and resistance antibiotic pattern among adult population in a university community. Multi staged random sampling was used to obtain up to 465 respondents. Clean catch mid-stream urine samples were collected and prepared. Culture was done in three different media for identification and isolation of bacteria. The antimicrobial sensitivity was determined using sensitivity disk by Kirby-Bauer method. Data was analyzed using SPSS version 25 at 95% confidence interval and a p-value of less than 0.05 was considered significant. Data was presented in frequencies and percentages as appropriate.

Results: Female respondents (76.97%) were more. 20-24 age group had the highest number of respondents (50.66%). 36.29% had bacteria growth in their urine. Escherichia coli (63.6%) is the commonest microbial agent found in the urine of the population, followed by *staph aureus* (20%), then *Klebsiella* (16.4%). The isolated organisms were mostly resistant to the common antibiotics. However, *E. coli* was slightly susceptible to Levofloxacine (37.1%), Ciprofloxacine (31.4%) and Gentamycin (30%). *Klebsiella* showed mild susceptibility to Levofloxacine (15%), Ciprofloxacine (12%) and Ceftriazone (9%).

Conclusion: *E.coli*, *staph aureus* and *Klebsiella* are the dominant microbial flora found while levofloxacin, ciprofloxacin, ceftriazone and augmentin are the most sensitive antibiotics.

Keywords: Microbial flora; Asymptomatic; Bacteriuria; Sensitive antibiotics; Heathy adult population; FUTO

1. Introduction

Urinary tract infection (UTI) is a general term for infections involving any part of the urinary tract namely the kidneys, ureters, bladder and urethra. UTI is known to be among the most common bacterial infections acquired in the community and in hospitals[1] and account for a large number of hospital visits worldwide[2]. In Nigeria, Jumbo and his colleagues[3] documented 12.3% and 9.3% prevalence of community acquired and nosocomial UTIs respectively. The

* Corresponding author: VITALIS OBISIKE OFURU

prevalence of UTIs in Nigeria, according to Akinsete and Ezeaka[4] also vary across geo-political zones with reported rates of 11.96% in South West-Nigeria, 2.7% in an urban setting in south east and 13.7% in Northern Nigeria.

Urinary tract infections are caused by both Gram-negative and gram-positive bacteria and these include: uropathogenic *E. coli, klebseilla prenumoniae*, *staphylococcus saprophyticus, enterococus faecalis*, group *B streptococcus* (GBS), *proteous mirabilis, pseudmonas aeroginosa and staphyloccus aureus*; Candida spp. which are fungal organisms also cause urinary tract infection[1]. Most of these pathogens are present naturally in the peri-urethral and perianal area, and ascend through the urethral orifice to cause UTI[1].

The diagnosis of urinary tract infection is based on symptoms and or results of investigations: symptoms depend on the part of the urinary tract involved, the causative organisms, the patient's ability to exert an immune response to it, the severity of the infection and age of the person; Symptomatic urinary tract infection can be acute or chronic, complicated or uncomplicated[5]. Symptoms may include fever, burning sensations while urinating, suprapubic pain and pyuria[5].

Asymptomatic bacteriuria is the presence of bacteria in the urine of an individual that does not have symptoms and signs of urinary tract infection, provided that the urine was collected without contamination[6-9]. Patients with asymptomatic bacteriuria show no obvious symptoms and appear clinically normal. Several researches have been conducted on asymptomatic bacteriuria in varying age-groups and cohorts[6-9]. Delzell and Lefevre[6] documented a global prevalence of 2 – 10%. In Nigeria, Olamijulo et al[7] documented a higher prevalence rate of 14.6%. Prevalence of 20-35% has been documented among women attending antenatal care in Ghana[8]. In children, a meta-analysis on prevalence of asymptomatic bacteriuria conducted by shakkih and others[9] showed a prevalence of 0.37% (95% CI 0.09 -6.82) in boys and 0.47% (95 CI 0.36 – 0.59) in girls. The sub group with the highest prevalence from their meta-analysis were uncircumcised males less than 1 year of age and females greater than 2 years of age. Other groups at high risk of asymptomatic bacteriuria include spinal cord injury patients on intermittent clean catheterization, patients with indwelling urethral catheter, diabetics, the elderly and institutionalized patients.

A common clinical dilemma is whether or not to treat patients with asymptomatic bacteriuria. Whereas clinical benefit has been demonstrated in treatment of pregnant women with asymptomatic bacteriuria[10], it has not been so with the other cohorts. In fact, Infectious Diseases Society of America (IDSA) guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults[11] advocates that screening for and treatment of asymptomatic bacteriuria should not be recommended for premenopausal non pregnant women, diabetic women, older persons living in the community, elderly institutionalized subjects, persons with spinal cord injury and catheterized patients while catheter remains in situ. This is because of increased incidence of adverse antimicrobial effects and possibility of reinfection with antibiotic resistant organisms.

Screening for asymptomatic bacteriuria should not be done if there is no intention to treat the subject but screening is indicated in two scenarios: where asymptomatic bacteriuria is associated with adverse outcomes and the intervention with antimicrobial treatment improves those outcomes and in a research study to further explore the biology or clinical significance of bacteriuria[11]

In this study therefore, we intend to determine the common microbiological flora and the sensitivity pattern in the urine of apparently normal sexually active adults in a university community. This knowledge could be useful in the determination of antibiotics to use in empirical treatment of clinical cases of urinary tract infections.

2. Material and methods

This research utilized cross-sectional survey design. The study was carried out between January and February 2022 among university students of Federal University of Technology Owerri (FUTO). FUTO is the foremost university of technology established in the South East Nigeria in the year 1980. It is a tertiary institution situated in Owerri West Local Government Area of Imo State bounded by four communities namely Ihiagwa, Ezi-Obodo, Obinze and Umuagwo. There are twelve (12) faculties in it namely the School of Agriculture and Agricultural Technology (SAAT), School of Basic Medical Sciences, School of Biological Sciences (SOBs), School of Engineering (SSET), School of Environmental Sciences (SOES), School of Health Technology (SOHT), School of Information and Communication Technology (SICT), School of Management Technology (SMAT), School of Physical Sciences (SOPS), School of Postgraduate Studies (SGPS) and Directorate of General Studies. Each of these facilities have a minimum of five Departments. Students study their respective courses in these departments. The students are over 22,000 in population. There are hostel accommodations within the campus for students which are provided by the university management but a reasonable number of students live off-campus within the neighboring communities. Sick students are treated in the university health centre which is located within the institution, whenever they fall ill.

The study was carried out at the Faculties and Departments of the institution. It utilized a self-administered questionnaire. The variables considered were age, sex, symptoms of urinary tract infection (for exclusion), antibiotic treatment (for exclusion), urinalysis, urine culture, sensitivity and resistance pattern.

The study population was limited to apparently normal individuals, who did not have any symptoms of urinary tract infection. This might be a source of bias.

The exclusion list included those who voluntarily opted out of the study, students who had taken antibiotics medication for any other disease condition in the past two weeks before sample collection and menstruating female students. Students who reported any symptom and signs of urinary tract infection were also excluded from the study. We issued sterile universal bottles to each student for collection of clean catch midstream urine specimens. They were educated on how to collect urine with as little contamination as possible. We thought female students to cleanse their vulva and around their urethral opening with clean water, and to part their vulva while passing urine in order to get clean catch urine. The containers were serially labelled and gender indicated. We did not write names to avoid bias and ensure confidentiality. The corresponding questionnaires from the respective students were also tagged with the same numbers labelled on their sterile bottles. We examined the collected clean catch mid-stream urine samples ~~were~~ macroscopically, microscopically, and chemically. We Cultured the urine in three different media for identification and isolation of bacteria. Materials used included Bunsen burner, wire loop, blood agar, chocolate agar, MacConkey agar, face mask, latex gloves. The universal bottle containing midstream urine was mixed by inverting the bottle. The lid was passed through the Bunsen flame three times to reduce the load of micro-organisms on it. The lid was opened and a loopful of urine was collected using the wire loop. The loopful was inoculated in the culture medium to make primary inoculum. The culture medium was then placed in the incubation chamber at 37^0C for 24 to 48hours for the growth of micro-organism. Urine culture was considered to have significant bacteriuria when colony forming units $\geq 10^5$/ ml of voided urine was obtained. A single pure colony was suspended in nutrient 6 and sub cultured in machinery plate and blood agar plate incubated at 37ºC for 24hour. Isolates were identified using colony morphology and biochemical test. The antimicrobial sensitivity was determined using sensitivity disk by Kirby-Bauer method.

The population of students in the institution was estimated to be 25,000.00. The formula for survey developed by Yaro Yamen was used to determine the minimum sample size as follows: n $= \frac{N}{1+Ne^2}$ where n = minimum sample size, N = Estimated Total population of students, and e = desired precision/level of significance, usually 5% (0.05) at 95% Confidence Interval (CI). Hence, n = 25,000/ 1+25,000 X 0.05^2 = 400.

Five hundred (500) students were selected for the study via random sampling technique. Multi staged sampling was done. In the first stage, the twelve faculties were sampled randomly and five of the faculties chosen. In the second stage, five departments were randomly selected from each of the faculties and in the final stage of sampling twenty (20) students from each department were selected giving a total of 100 students in each faculty. We had a meeting with the respective department heads and student representatives who made the students available on agreed dates. The final selection of the 20 students from each department was done through balloting. Each of the selected student was given a structured questionnaire. The questionnaire was pretested prior to the study to ensure accuracy, appropriateness and to ascertain it was understandable. The collected data were checked for consistency and accuracy. The sterility of the prepared culture was checked by incubating 5% of the prepared culture randomly selected for 24hours at 37ºC. Laboratory identification procedure such as inoculation of culture media, colony characterization and measuring of antibiotic susceptibility were checked. Data was analyzed using SPSS version 25. All analysis was done at 95% confidence interval and a p-value of less than 0.05 was considered significant. Data was presented in frequencies and percentages as appropriate. The association of bacteria and demographic characteristics was assessed using the chi-square statistic.

3. Results

Table 1 Demographic characteristics

	Frequency(n=456)	Percent(%)
Gender		
Male	105	23.03
Female	351	76.97
Age-Groups(years)		
15-19	57	12.50
20-24	231	50.66
25-29	126	27.63
30-34	33	7.24
35-39	9	1.97

The table shows that the number of female respondents (351, 76.97%) was more than the male respondents (105, 23.03%). Also, 20-24 age group had the highest number of respondents (50.66%)

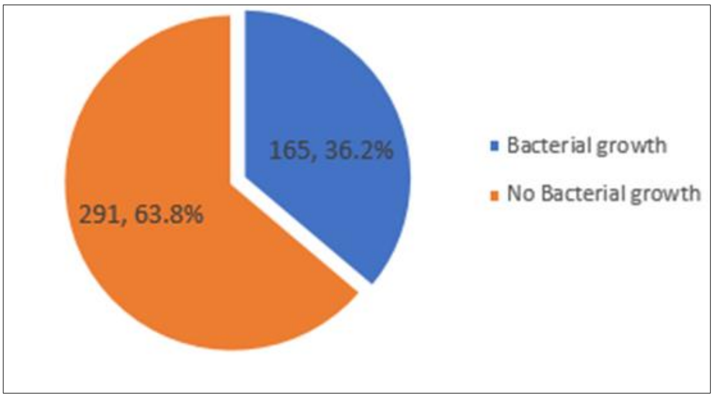

Figure 1 Prevalence of Bacteria growth on culture

The figure shows that 165 (36.29%) respondents had bacteria growth in their urine while 291 (63.8%) had no bacteria growth.

Table 2 Association of Age groups and bacteriuria

Age-group(years)	Bacteriuria n(%)	No Bacteriuria n(%)	Total n, (%)	Chi-square(p-value)
15-19	33(57.89)	24(42.11)	57(100.0)	24.76 (0. 015) *
20-24	60(25.97)	171(74.03)	231(100.0)	
25-29	63(50.00)	63(50.00)	126(100.0)	
30-34	6(18.18)	27(81.82)	33(100.0)	
35-39	3(33.33)	6(66.67)	9(100.0)	

Statistically significance (p<0.05)

The table showed that the proportion of bacteriuria was significantly higher (p = 0.015) among the age group of 25-29 in comparison to other age groups. Hence, the occurrence of bacteriuria was significantly associated with the age group 25-29 among the study population.

Table 3 Distribution of Bactria culture

Bacteria isolated	Frequency(n=165)	Percent (%)
Escherichia coli	105	63.6
Staphylococcus aureus	33	20.0
klebsiella	27	16.4

The table shows that *Escherichia coli* (63.6%) is the commonest microbial agent found in the urine of the population, followed by *Staph aureus* (20%), then *Klebsiella* (16.4%).

Table 4 Antibiotic susceptibility pattern of *Escherichia coli* Isolate

Escherichia Coli			
Antibiotics	**Sensitive (%)**	**Resistant (%)**	**Total (%)**
Augmentin	15(14.3)	90(85.7)	105(100.0)
Ceftriazone	21(20.0)	84(80.0)	105(100.0)
Ciprofloxacine	33(31.4)	72(68.6)	105(100.0)
Levofloxacine	39(37.1)	66(62.9)	105(100.0)
Amoxycilline	9(8.6)	96(91.4)	105(100.0)
Gentamycin	30(28.6)	75(71.4)	105(100.0)
Nalidixic Acid	3(2.9)	102(97.1)	105(100.0)
Streptomycin	3(2.9)	102(97.1)	105(100.0)
Ampicillin	3(2.9)	102(97.1)	105(100.0)

The table shows that the *E. coli* isolated was mostly resistant to most of the common antibiotics. However, it was slightly susceptible to Levofloxacine (37.1%), Ciprofloxacine (31.4%) and Gentamycin (30%).

Table 5 Antibiotic susceptibility of Klebsiella organism

Klebsiella organism			
Antibiotics	**Sensitive (%)**	**Resistant (%)**	**Total (%)**
Augmentin	2(22.2)	21(77.8)	27(100)
Ceftriazone	9(33.3)	18(66.7)	27(100.0)
Ciprofloxacine	12(44.4)	15(55.6)	27(100.0)
Levofloxacine	15(55.6)	12(44.4)	27(100.0)
Amoxycilline	6(22.2)	21(77.8)	27(100.0)
Gentamycin	6(22.2)	21(77.8)	27(100.0)
Nalidixic acid	0(0.00)	27(100.0)	27(100.0)
Streptomycin	0(0.00)	27(100.0)	27(100.0)
Ampicillin	3(11.1)	24(88.9)	27(100.0)

The table shows that the Klebsiella organism was mainly resistant to most of the common antibiotics but showed mild susceptibility to Levofloxacine (15%), Ciprofloxacine (12%) and Ceftriazone (9%).

Table 6 Antibiotic susceptibility of Staphylococcus aureus

	Staphylococcus aureus		
Antibiotics	**Sensitive (%)**	**Resistant (%)**	**Total (%)**
Augmentin	12(36.6)	21(63.6)	33(100.0)
Ceftriazone	15(45.5)	18(54.5)	33(100.0)
Ciprofloxacine	12(36.4)	21(63.6)	33(100.0)
Levofloxacine	18(54.5)	15(45.5)	33(100.0)
Amoxycillin	6(18.2)	27(81.8)	33(100.0)
Gentamycin	15(45.5)	18(54.5)	33(100.0)
Nalidixic acid	0(0.00)	33(100.0)	33(100.0)
Streptomycin	0(0.00)	33(100.0)	33(100.0)
Ampicillin	0(0.00)	33(100.0)	33(100.0

The table shows that staphylococcus organisms showed sensitivity as compared to resistance to Levofloxacine (54.5%), Ceftriazone (45.5%), Ciprofloxacine (36.4%), and Augmentin (36.4%).

4. Discussion

The urinary tract which extends from the kidney to the urethral meatus and is usually sterile, has mechanisms that prevent infections, and the mechanisms include; acidity of urine, mucosal barriers, immunologic barriers and complete emptying of the urinary bladder during micturition[12]. Some factors like pregnancy, diabetes and bladder outlet obstruction may cause immunologic, hormonal and mechanical changes that predispose to stasis and or urinary reflux resulting in the presence of bacteria in urine.[13,14]

Routine culture techniques may yield growth of micro-organisms in some people who do not have symptoms of UTI, and expanded quantitative urine culture and sequencing techniques have identified bacterial growth in urine of healthy persons in whom routine urine culture did not yield growth of any organism; these urinary microbiomes are presumed to be responsible for sporadic UTI[15]. Accordingly, some experts have suggested using the term '' urinary tract dysbiosis'' in place of UTI because most pathologic bacteria are native to the urinary tract and their virulence may have been occasioned by a variation in the microbiota of the urinary tract[16]. The diagnosis of UTI which is based on Kass criterion propounded by Edward Kass in 1957 is premised on counting bacteria in fresh urine and urine bacteria count of one hundred thousand colony forming units or more is considered UTI[17].

In this study 351 (76.97%) of the respondents were female while 105 (23.03%) were male. A study in Ogun state, Nigeria by Agu et al[18] also had more female respondents compared to male. The age group with the highest frequency was 20-24 (table 1) and a similar age group had the highest frequency in the study by Agu et al[18]. This may be due to the fact that both studies were carried out in tertiary institutions and may indicate that most students in tertiary institutions in Nigeria fall within that age group.

A significant number, 165(36.29%) of those tested had bacterial growth. This is similar to what was found by other researchers[18,19]. Recent and better methods of culturing bacteria in urine have shown that most people have bacteria in their urine and in fact even a wider spectrum[18,19,20]. In a study by Anderson et al[21] it was discovered that there were uncultured bacteria in women without symptoms of UTI. Similarly, Wolfe et al[22] used high- through put sequencing techniques to identify bacteria in patients without UTI. These may indicate that the number of people with no bacterial growth in our study may be lower if subjected to further testing. However, these advanced urine tests to identify bacteria are not routinely done because the IDSA does not recommend them[9] and only pregnant women and those with significant risk of infection are treated for asymptomatic bacteriuria.[8] However, as evidence show that the micro-organisms that cause UTI may have been in the urine long before the onset of sypmtoms[15,16,19], a good knowledge of the type of bacteria present in urine is necessary and will help guide empirical treatment of UTI.

The age group with the highest number of people with bacteria growth in their urine was 25-29 years. This was statistically significant with a p value of 0.015 (table 2). Odoki et al[23] in their study found that the prevalence of UTI was highest in the age group 20-29. This was similar to what we found in our study. The prevalence of UTI increases with age[24] but the study population were students of a university where most students are young. Aging is associated with hormonal and mechanical changes that may predispose to UTI, however in the young, the peak of uncomplicated UTI correlates with maximum sexual activity between the ages of 18 and 39 years.[25] This may mean that those between the ages of 25-29 in this study have the maximum sexual activity because this study was carried out among sexually active students.

The distribution of the most bacteria cultured as shown in table 3 were E coli 105(63.6%), S. aureus and Klebsiella spp. Schmiemann et al[26] also reported that E. coli caused most of the cases of complicated and uncomplicated UTI. In a study among pregnant women in Pakistan by Tabinda et al[27] the most common cause of asymptomatic bacteriuria was also E. coli followed by Klebsiella and Staphylococcus spp. These are similar to what was found in our study. Other studies by Agu et al[18], and Bhayani et al[28] also found E. coli to be the most common cause of asymptomatic bacteriuria.

The sensitivity pattern showed that E. coli was most sensitive to Levofloxacin, ciprofloxacin and gentamycin as shown in table 4. This was similar to studies by Agu et al[18]. Oluwole et al[29] reported fluroquinolones had the highest sensitivity patterns to E coli. Klebsiella and S. aureus were also sensitive to Levofloxacin and ciprofloxacin (tables5 and 6). These medications may be used for empiric treatment of UTI while waiting for the results of urine culture.

5. Conclusion

The knowledge of the types of micro-organisms that cause asymptomatic bacteriuria is important because similar organisms cause symptomatic UTI. The sensitivity of these organisms to antibiotics helps guide presumptive use of antibiotics in treatment of UTI. The most common organism implicated in asymptomatic bacteriuria is E. coli. The commonly culture organisms were most sensitive to levofloxacin and ciprofloxacin.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

Approval for this study was received from the ethical committee of the institution.

Statement of informed consent

All patients recruited for the study gave written consent before their data and samples were collected. Results of the urine culture were not disclosed to any other person.

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