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Research Article

Diversity and Antibiotic Susceptibility of Some Bacterial Uropathogens among Female Students of a Tertiary Institution in South-Eastern Nigeria

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ABSTRACT

Urinary Tract Infection (UTI) is one of the common diseases that affect people of all age groups. It can be symptomatic or asymptomatic but the prevalence is higher in females. This study investigated the diversity and antibiograms of the implicated microorganisms among female undergraduate students of Michael Okpara University of Agriculture, Umudike. A total of 60 healthy female students of the university who consented to the study were included. Each participant submitted a 15ml urine sample and records of age and place of residence were taken. The samples were cultured on Blood agar and MacConkey agar. Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method. Significant bacteriuria was observed in 23(38.3%) of the samples; while insignificant bacteriuria was 22(36.7%) and there was no bacterial growth in 15(25%). The prevalence of significant bacteriuria was higher among female students who stayed in the hostel 19(82.6%) than among those who stayed off-campus 4(17.4%). Significant bacteriuria (69.6%) occurred highest among those between 22-24 years old, followed by those within the age group 25-27 years (17.4%). The Gram-positive isolates were *Enterococcus faecalis* and *Staphylococcus saprophyticus*; while Gram-negative isolates were *Escherichia coli, Klebsiella* species and *Proteus* species The Gram-positive isolates were highly susceptible to Pefloxacin, Gentamicin and Zinnacef; while the Gram-negative isolates were highly susceptible to Ciprofloxacin, Pefloxacin and Ofloxacin. The findings emphasize the need for personal hygiene among the female undergraduates, proper sanitation, and provision of sufficient standard toilet facilities in the hostels.

Keywords: Urinary Tract Infections; Antibiotic susceptibility; Uropathogens; Bacteriuria; Female students

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INTRODUCTION

Urine is commonly regarded as sterile, devoid of microorganisms, with potential sources of infection typically arising from the urethra, which serves as the primary site for initiating infections (Siiri *et al.*, 2009).

Urinary tract infection is any infection that occurs along the length of the urinary tract. It is characterized by the presence of bacteria resulting in an increased bacterial load (often greater than 10^5CFUmL) in the urine sample (Vasudevan *et al.,* 2014*)*. Also, according to the National Institute for Health and Clinical Excellence (NIHCE) guidelines, urinary tract infection is defined by a combination of clinical features and the presence of bacteria and or fungi in urine (NIHCE, 2007).

Urinary tract infections (UTIs) are primarily bacterial in nature, although involvement of fungi and viruses is also possible (Siiri *et al*., 2009). Urinary tract infections (UTIs) are highly prevalent and impact a significant portion of global population. Annually, approximately 150 million individuals worldwide are affected by UTIs. In the United States alone, UTIs incur an estimated annual cost of $5 billion (Flores-Mireles, 2015; McCann *et al.,* 2020). The predominant organisms responsible for UTIs are members of the Enterobacteriaceae, with *E. coli* accounting for over 80% of the cases (Derbie *et al*., 2017; Mwang'onde and Mchami 2022). This is followed by *Staphylococcus saprophyticus* which accounts for 5–15% of UTIs (Michael *et al.*, 2007). In addition, other

bacterial genera implicated include, *Klebsiella, Proteus, Pseudomonas* and *Enterobacter* (Derbie *et al*., 2017)

Gram-positive bacteria such as *Staphylococcus aureus* and Coagulase-negative Staphylococci (CoNS) are commonly associated with community-acquired UTIs, however UTIs caused by fungi and viruses are rare; but yeasts notably *Candida albicans*, can occasionally be isolated from catheterized or immunocompromised patients (Vasudevan *et al*., 2014).

The majority of UTIs occur in women due to some anatomical and physiological factors. These include the shorter length of the female urethra compared to males, which increases the risk of bacterial migration into the bladder. Additionally, the proximity of the anus to the female urethra facilitates the transfer of bacteria from the gastrointestinal tract. Changes in vaginal pH, often due to decreased levels of commensal bacteria, and hormonal fluctuations during menstrual cycles or pregnancy can also predispose women to UTIs (Koffi *et al*., 2020).

UTI can be asymptomatic or symptomatic and it is characterized by a wide range of symptoms from mild voiding irritation to bacteremia, sepsis, or even death. Infection of the urinary tract could manifest differently depending on the site of the infection and the length of time involved (Takhar and Moran 2014). Infections that affect the lower urinary tract called cystitis involve the bladder alone with symptoms which include painful urination, burning sensation, frequent urination, and/or urge to urinate. Other infections that affect the upper urinary tract referred to as pyelonephritis involve the kidneys and other organs; the symptoms include fever, and flank pain during urination in addition to those of the lower urinary tract.

Culture of urine is usually the first step towards diagnosis when UTIs are suspected. Urine culture is traditionally the gold standard for diagnosis of UTIs and cultures without microbial growth essentially rules out UTI caused by the most common organisms. Test accuracy (sensitivity and specificity) depends on the colony count threshold and specimen collection method (Gibson and Toscano, 2012). Urine microscopy is a pre-test to look out for UTI and involves detection of the presence of red blood cells, pus cells, bacteria, parasites, and other components.

Drug resistance among bacteria causing UTI has increased since the introduction of UTI chemotherapy thereby complicating the clinical management of UTIs (Donkor *et al*., 2019). Consequently, this has made susceptibility testing particularly important. Antibiotic sensitivity can be tested with these cultures, making them useful in the selection of the appropriate antibiotic treatment. When UTI is not handled promptly, can lead to colonization of the kidneys by the infecting organisms, resulting in kidney damage and increased economic burden. Consequently, this work was therefore carried out to determine the diversity and antibiogram of uropathogens among undergraduate students living in school hostels and off-campus of a tertiary institution in South-East of Nigeria.

MATERIALS AND METHODS

Study Area

The work was carried out in Michael Okpara University of Agriculture, located along Ikot-Ekpene road, Umudike, in Ikwuano Local Government Area of Abia State, Nigeria.

Study Design

This was a cross-sectional study involving apparently healthy students who were recruited by simple random technique. Verbal informed consent was obtained from the students and simple questionnaire used to obtain data including age, hostel, medical history etc. Only students who satisfied the selection criteria of not been on antibiotics preceding one week of sample collection were enrolled after clear instructions on how to collect urine samples by cleaning the genitalia before voiding of urine.

Collection of Urine Samples

The participants were instructed to provide about 15mL of fresh mid-stream urine into a sterile universal container. Each sample was appropriately labelled, including age, room number, hostel, and the date and time of collection. A total of 60 samples (20 samples from those living outside the hostel and 40 samples from those living in the hostel) were collected from female students of Michael Okpara University of Agriculture Umudike (MOUAU). The samples were analysed in the Microbiology laboratory of MOUAU.

Microscopy

Each urine sample (15ml) was transferred aseptically into a centrifuge tube and spun in the centrifuge at 3000 rpm for 5 minutes. The supernatant was decanted leaving the sediment at the bottom of the tube. A drop of the sediment was pipetted and placed on a microscope slide, then covered with a coverslip. Examination was done using 10x and 40x objectives of the compound microscope for eggs of *Schistosoma haematobium, Trichomonas vaginalis,* pus cells, red blood cells, casts, yeast cells, and epithelial cells. The pus cell counts are usually reported as: Few: - 0-10 white blood cells/HPF (High Power Field), 11-40 WBC/HPF (moderate), and more than 40 WBC/HPF (many). Counts above 40 indicates urinary tract infections.

Culture

Each of the urine samples was homogenized thoroughly by rotating the container clockwise and anticlockwise directions. The urine samples were collected using a flamed calibrated wire loop. The loopful urine was streaked peripherally over the surface of the dried MacConkey agar and on Blood agar. All plates were incubated at 37°C aerobically for 24hr. The plates were examined macroscopically for growth. The bacterial colonies were counted and multiplied by 100 to give an estimate of the number of bacteria present per millimetre of urine. A significant bacterial count was taken as any count equal or over 10, 0000 (10^5) CFU/ml. Pure cultures were obtained by repeated streaking. Bacterial isolates were identified based on a combination of cultural, morphological and biochemical characteristics as described by (Cheesbrough, 2001).

Antimicrobial Susceptibility Testing

The antibiotic susceptibility tests against the isolates were done using the standard disc diffusion method as described by Kirby–Bauer. (CLSI, 2009). This was carried out using commercial discs containing the following antibiotics: Septrin (30µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Ciprofloxacin (10µg), Amoxacillin (30µg), Gentamicin (10µg), Pefloxacin (10µg), Streptomycin (30µg), Tarivid (10µg), Augmentin (30µg) Erythromycin (10µg), Amplicox (30µg), Zinnacef (20µg), Rocephin (25µg). The nutrient broth was prepared according to the manufacturer's specification and 10ml was dispensed into sterile test tubes. A sterile loop was used to pick a pure colony of the test isolate and emulsified in the broth. The broth was then incubated at 37°C for 18-24 hours. The turbidity of the suspension was measured and adjusted to the density of 0.5 McFarland in order to standardize the inoculum size. The isolate was streaked on sterile Mueller Hinton agar plates aseptically using sterile swab sticks. The antibiotic discs were placed on the inoculated Mueller-Hinton plates with the aid of a sterile forceps, at equal distance from each other and then gently pressed down onto the surface of Mueller-Hinton agar to ensure complete contact. The plates were inverted and incubated at 37°C for 24 hours. The zone of inhibition that developed around each disc was measured from underneath each plate with the aid of a ruler and recorded in millimetres (mm).

RESULTS

Out of the sixty (60) samples examined, 23(38.3%) showed significant bacteriuria, 22(36.7%) had insignificant bacteriuria and 15(25%) had no growth as shown in Table 1.

Table 2 shows the Diversity of uropathogens among the female students used for the study. It shows that out of the 23 subjects with significant asymptomatic bacteriuria, *Escherichia coli* was the most frequently occurring with 12(52.2%); followed by 5(21.7%) recorded for *Enterococcus faecalis*, 2(8.7%) for *Klebsiella* species*,* 2(8.7%) for *Proteus* species and 2(8.7%) for *Staphylococcus saprophyticus.* Of the 23 subjects with bacteriuria, 19(82.6%) were hostel occupants; while 4(17.4%) stayed off-campus.

Table 3 shows the age distribution of significant bacteriuria among the study subjects. The data reveals that 23 subjects out of a total of 60 individuals had significant bacteriuria, with 16(69.6%) found in the age group 22-24, 2(8.7%) found in the age group 19-21, 4(17.4%) found in the age group 25-27 and 1(4.3%) found in the age group 28-30 years old.

The antibiotic sensitivity profile of the isolates is shown in Tables 4 and 5. The Gram-positive isolates were resistant to Erythromycin, Ciprofloxacin, Ampiclox, Amoxacllin, Rocephin, Septrin and Streptomycin. *Enterococcus faecalis* was highly susceptible to Pefloxacin, *Staphylococcus saprophyticus* was susceptible to Gentamicin and Zinnacef. Of the Gram-negative isolates, *Proteus* and *Klebsiella* were highly resistant to Septrin, Chloramphenicol, Amoxicillin and Augmentin. All of the Gram-negative isolates were highly susceptible to Pefloxacin, Ciprofloxacin, followed by Ofloxacin. *Escherichia coli* was susceptible to all the antibiotics, with the most being Ciprofloxacin (100%) and Pefloxacin (100%), the least being Amoxicillin, Septrin, Chloramphenicol and Augmentin (6.2%). The isolate was moderately susceptible to Gentamicin (41.7%). *Proteus* was moderately susceptible to Sparfloxacin (50%) and Gentamicin (50%). *Klebsiella* was moderately susceptible to streptomycin (50%).

Table 1: Occurrence of UTIs among the study subjects

Samples obtained	No of occurrence	Percentage of occurrence (%)
Positive samples (Significant bacteriuria)	23	38.3
Negative samples (No significant bacteriuria)	22	36.7
Samples with no growth	15	25
Total	60	100

Table 2: Diversity of bacterial uropathogens among the female undergraduates

Pathogen isolated	% Occurrence	No of positive culture	No of infected students living in the hostel	No of infected students living in the off-campus
Escherichia coli	52.2	12	9	3
Enterococcus faecalis	21.7	5	4	1
Klebsiella species	8.7	2	2	0
Proteus species	8.7	2	2	0
Staphylococcus saprophyticus	8.7	2	2	0
Total	100	23	19(82.6)	4 (17.4)

Table 3: Age-distribution of UTI among the female students of Michael Okpara University of Agriculture, Umudike

Age distribution	No of females examined	No of positive culture	% occurrence
19-21	7	2	8.7
22-24	42	16	69.6
25-27	8	4	17.4
28-30	3	1	4.3
Total	60	23	100

Table 4: Antibiotics sensitivity of the Gram-positive isolates from urine culture

Isolates	E	PEF	CPX	CN	APX	Z	AM	R	S	SXT
S. saprophyticus	0	0	0	2	0	2	0	0	0	0
N=2	(0.0)	(0.0)	(0.0)	100	(0.0)	100	(0.0)	(0.0)	(0.0)	(0.0)
E. faecalis	0	5	0	0	0	0	0	0	0	0
N=5	(0.0)	100	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)

Keys: E= Erythromycin, PEF= Pefloxacin, CPX= Ciprofloxacin, CN= Gentamicin, Z= Zinnacef, AM= Amoxacillin, R= Rocephin, S= Streptomycin, SXT= Septrin

Table 5: Antibiotic sensitivity of the Gram-negative isolates from urine culture

Isolates	SXT	CH	SP	CPX	AM	CN	PEF	S	OFX	AU
E. coli	1	1	2	12	1	5	12	2	8	1
N=12	(8.3)	(8.3)	(16.7)	(100)	(8.3)	(41.7	(100)	(16.7)	(66.7)	8.3)
Proteus species	0	0	1	2	0	1	2	0	2	0
N=2	(0.0)	(0.0)	(50)	(100)	(0.0)	(50)	(100)	(0.0)	(100)	(0.0)
Klebsiella species	0	0	0	2	0	0	2	1	2	0
N=2	(0.0)	(0.0)	(0.0)	(100)	(0.0)	(0.0)	(100)	(50)	(100)	(0.0)

Keys: SXT= Septrin, CH= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AM= Amoxacillin, CN= Gentamicin, PEF= Pefloxacin, S= Streptomycin, OFX= Ofloxacin, AU= Augmentin

DISCUSSION

Bacteriuria occurs as a result of colonization by species of bacteria that cause UTIs and different microbes have been implicated in this (Ugwu *et al*., 2019). Bacterial growth was not detected in 15(25%) of the processed samples which was confirmed by microscopic examination showing fewer than 5 pus cell count thereby establishing it as non-significant (Otajevwo and Eriabor, 2014). The 38.3% occurrence of significant bacteriuria among the female students is similar with rates reported by some authors (Anigilaje and Bitto, 2013; Otajevwo, 2014). Other authors recorded lower UTI prevalence rates of 4.5% (Jurbe *et al.,* 2023) and 4% (Ugwu *et al.,* 2019). Higher prevalence rates have been reported by some authors (Agu *et al.,* 2014; Onuoha and Fatokun, 2014). The high or low incidence rates may be attributed to poor environmental conditions where the subjects reside in terms of lack of proper personal and environmental hygiene, sexual promiscuity etc. among the Nigerian men and women (Akinyemi *et al.,* 1997).

Five uropathogens were isolated in this study, of which Gram-positive bacteria constituted 31.4% and Gram-negative bacteria constituted 69.6%, this agrees with the report of Otajevwo and Eriabor (2014). The two most occurring uropathogens isolated in this study were *Escherichia coli* (52.2%) and *Enterococcus faecalis* (21.7%). Other uropathogens isolated were *Klebsiella*

species (8.7%), *Proteus species* (8.7%) and *Staphylococcus saprophyticus* (8.7%). The occurrence of *Escherichia coli* and *Enterococcus faecalis* as the most commonly occurring uropathogens is consistent with the report of a previous study by (Anigilaje and Bitto, 2013). This present result however, does not agree with the results of other studies (Onuoha and Fatokun, 2014; Haruna *et al.,* 2019) as the most and second most occurring uropathogens in urinary tract infection.

The higher occurrence of *Escherichia coli* (52.2%) agrees with other reports that indicated that *Escherichia coli* was the commonest pathogen causing UTIs in females (Mbata, 2007). This predominance of *Escherichia coli* could be attributed to the fact that it is a commensal of the bowel and that infection is mostly by faecal contamination due to poor hygiene (Njoku, 2001), and the predilection of the organisms from the toilets and shortness of the female urethra (Nicolle, 2002).

UTI prevalence rate was higher in female students living in the hostel (82.6%) than in the female students living off campus (17.4%) of which *Escherichia coli* and *Enterococcus faecalis* occurred more in the female students living in the hostel. The difference in prevalence may result from different environmental conditions and host factors and practices such as hygiene practices. There is also a possible link between the occurrence of UTIs among the female students living in the hostel and the level of personal hygiene or the state of toilet facilities in the hostels. Most of the students examined rated the hostel toilets as bad. When the toilets are dirty there is an accumulation of urine sediments forming a thick scum, in this case students could become infected during urination.

Infection occurred highly in females between the ages of 22-24 and 69.6% of the positive culture falls within this bracket, followed by 25-27 age group (17.4%) and none fell under the age group of 16-18. This finding agrees with the reports of some authors which stated that urinary tract infection is most prevalent during youth and adulthood as indicated by the 22-24 age group occurring as the most implicated in urinary tract infection in this study (Mbata, 2007; Nsofor *et al.,* 2016). This age group as well as 25-27 consists of young people who are characteristically vulnerable to increased sexual activities that predispose them to urinary tract infection (Oladeinde *et al.,* 2011).

Antibiotic sensitivity showed that he Gram-positive isolates were resistant to Erythromycin, Ciprofloxacin, Ampiclox, Amoxicillin, Rocephin, Streptomycin and Septrin. The most active antibiotics against the Gram-positive isolates were Pefloxacin (100%) against *Enterococcus faecalis*, Gentamicin (100%) and Zinnacef (100%) against *Staphylococcus saprophyticus*. A remarkable result was obtained with Ciprofloxacin and Pefloxacin, followed by Ofloxacin which although performed poorly against the Gram-positive isolates. *Escherichia coli* showed susceptibility to all the antibiotics used but highly susceptible to Ciprofloxacin (100%), Pefloxacin (100%) and Ofloxacin (66.7%). Antibiotic resistance has been attributed to high prescription rates of these antibiotics since they are relatively cheaper and easily available. In addition, widespread inappropriate use of antimicrobial agent in Nigeria is also due to the possibility of buying antibiotics from pharmacy stores with or without prescription, this is a significant factor contributing to the development of resistance to antimicrobial agents (Manga *et al.,* 2021). The prevalence of multiple antibiotic-resistant strains in this study is a possible indication that very large population of bacterial isolates has been exposed to several antibiotics (Oladeinde *et al.,* 2011).

Three antibiotics, Ciprofloxacin, Pefloxacin and Ofloxacin were found to be more effective for all the Gram-negative isolates in this study particularly *Escherichia coli, Proteus* species and *Klebsiella* specie, while Gentamicin and Zinnacef were found to be more effective against *Staphylococcus saprophyticus.*

CONCLUSON AND RECOMMENDATIONS

An occurrence of 38.3% significant bacteriuria recorded in this study, indicates that UTI may be a health problem among female undergraduates of Michael Okpara University of Agriculture, Umudike (MOUAU). *Escherichia coli* was implicated as the most common causative agent, Pefloxacin, Ciprofloxacin and Ofloxacin were the most sensitive antibiotics against the bacteria isolates.

There is need for the education of the students on the importance of personal hygiene as most of the implicated isolates are commensals of perianal and vaginal regions.

Adequate health facilities should be provided for the university community and environs in order to tackle the menace of UTI among female population. The study population should be discouraged from the abuse of antibiotics as this leads to drug resistance by bacteria. Periodic epidemiological studies such as this study will also help in identifying the important UTI pathogens with a view to developing an effective and proper treatment model.

Conflicts of interest: None

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