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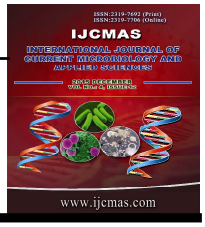

Original Research Article

Isolation, Identification and Antibiotic Sensitivity Pattern of Bacteria from Urine Samples of Female Students Living in the Hostels of Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State, Nigeria

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Keywords

Urinary tract infections, Female students, Gram negative bacteria, Antibiotics, Sensitivity

A B S T R A C T

Urinary tract infections (UTIs) can be complicated if not diagnosed early and treated. Bacteriological analysis of urine samples collected from female students staying in female hostel of Chukwuemeka Odumegwu Ojukwu University, Uli Campus was carried out. A total of 100 urine samples were collected from the students, classified and analyzed for urinary tract infection (UTI) using pour plate method. The bacterial organisms isolated from the urine samples were characterized and identified using their colony descriptions, morphological and biochemical characteristics. The isolates were subjected to sensitivity test against conventional antibiotics using disc diffusion method. The bacteria isolated from the urine samples include *Escherichia coli* 53(56.38%), *Staphylococcus aureus* 11(11.70%), *Staphylococcus saprophyticus*19(20.21%), *Proteus mirabilis* 4(4.26%) and *Klebsiella pneumoniae* 7(7.45%). The results of the study revealed that 29.00% of the samples analyzed were positive for UTI (10^5 CFU/ml), 34.00% suspected for UTI (10^2-10^4 CFU/ml) and 37.00% were normal (below 10^2 CFU/ml). Significant UTI was seen most among students of age 20-24 years (37.88%), single females (30.85%) and students studying non medical related courses (48.48%). Most of the conventional antibiotics were active against the isolates, of which ciprofloxacin showed the highest inhibition zone diameter (18-26mm) against Gram positive and Gram negative isolates. However, the two Gram positive bacteria (*Staphylococcus aureus* and *Staphylococcus saprophyticus* were resistant to bacitracin, vancomycin, levofloxacin, amikacin and azithromycin. From this study, periodic testing of UTI and also sensitivity testing prior to treatment is advocated. Those found to be infected need to be treated with antibiotics like ciprofloxacin to avoid complications.

Introduction

Urinary tract infections (UTIs) are infections of the kidney, ureter, bladder or urethra

caused by bacteria which could be symptomatic or asymptomatic. Lower urinary tract infection is also referred to as a bladder infection. The most common symptoms are burning with urination and having to urinate frequently (or an urge to urinate) in the absence of vaginal discharge and significant pain—(Nicolle, 2008). These symptoms may vary from mild to severe (Lane and Takhar, 2011) and in healthy women last an average of six days (Colgan, 2011). Symptoms of an upper urinary tract infection or pyelonephritis, such as flank pain, fever, or nausea and vomiting in addition to the classic symptoms of a lower urinary tract infection, bloody urine or visible pus in the urine have been documented (Lane and Takhar,2011; Salvatore *et al*., 2011).

If the urine contains significant bacteria but there are no symptoms, the condition is known as asymptomatic bacteriuria (Lane and Takhar, 2011). The microbiology definition is usually greater than or equal to 10^5 colony forming unit per milliliter of the organism in two consecutive urine specimens (Rubin *et al*., 1992). According to Nicolle (2003), the frequency and natural history of asymptomatic bacteriuria vary from different populations. Bacteria isolated from the urine of patients with asymptomatic bacteriuria usually originate as colonizing flora of the gut, vagina or periurethral area (Nicolle, 2003). For patients subjected to urinary tract instrumentation, bacteria that contaminate urologic fluids may be introduced into the genitourinary tract. Without prior host colonization, the normal genitourinary tract is sterile, apart from the distal urethra.

Asymptomatic bacteriuria occurs following ascension of bacteria up the urethra into the bladder, sometimes with subsequent ascension to the kidneys. Organisms then persist in the urinary tract without eliciting a host response sufficient to produce symptoms or cause irritation (Nicolle, 2003). Variables such as host genetic predisposition, incomplete bladder emptying or the presence of a foreign body may all facilitate persistent UTI (Nicolle, 2003).

Urinary tract infections are the most frequent bacterial infection in women (Colgan, 2011).They occur most frequently between the ages of 16 and 35 years, with 10% of women getting an infection yearly and 60% having an infection at some point in their lives (Nicolle.2003; Salvatore *et al*., 2011). Women are more prone to UTIs than men because, in females, the urethra is much shorter and closer to the anus (Dielubanza and Schaeffer, 2011). As a woman's estrogen levels decrease with menopause, her risk of urinary tract infections increases due to the loss of protective vaginal flora (Dielubanza and Schaeffer, 2011).

Escherichia coli is the cause of 80–85% of community-acquired urinary tract infections, with *Staphylococcus saprophyticus* being the cause in 5–10%(Nicolle, 2003). It is believed that the bacteria are usually transmitted to the urethra from the bowel, with females at greater risk due to their anatomy. After gaining entry to the bladder, *E. coli* are able to attach to the bladder wall and form a biofilm that resists the body's immune response (Salvatore *et al*., 2011).

The mainstay of treatment of urinary tract infections is antibiotics. However, increasing antibiotic resistance is causing concern about the future of treating those with complicated and recurrent UTI (Pallett and Hand, 2010; Shepherd and Pottinger, 2013).Among uropathogens the rate of resistance is high and frequency of resistance to antibiotics and drugs is directly

linked to consumption of antibiotics (Goossens and Sprenger, 1998). It is thus, very important to carry out antibiotic sensitivity testing prior to treatment, so as to ensure the efficacy of the antibiotics prescribed. This study was carried out to determine the antibiotic sensitivity pattern of bacterial isolates from urine samples of female students living in the hostels of Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State, Nigeria.

Materials and Methods

Study Site

This study was conducted in female hostel, among the female students of Chukwuemeka Odumegwu Ojukwu University (formerly called Anambra State University), Uli Campus, Anambra State, Nigeria. This hostel is located near the university's medical centre, and the population of female students is higher than the required number of apartment per student. This makes the sanitary level of the environment to be below the stipulated standard.

Study Population

Urine samples were collected from the female students, with age ranging from 18 to 26 years. The collections were randomly selected on everyday basis within the periods of 6.30am to 8.00am. Those students who were on antibiotic treatment prior to the sampling period were excluded from the study. The urine samples collected were classified based on age, marital status and field of study.

Sample Collection

The students were properly educated on how to collect clean catch midstream urine samples with wide mouthed sterile capped containers after proper cleaning of the external genitalia. A total of one hundred (100) clean catch midstream urine samples were collected from the students. The urine samples were labeled and immediately sent to the laboratory for analysis (Obirikwurang *et al.,* 2012).

Culturing of the Samples

The urine samples were cultured using pour plate method (1.0 ml) on Nutrient agar (for total heterotrophic aerobic bacteria count), MacConkey agar (for Enterobacteriacea family) and Mannitol Salt Agar (For *Staphylococcus* species). Inoculated plates were incubated inverted at 37°C aerobically for 24 hrs. After incubation, the total heterotrophic aerobic bacterial counts were carried out, and then the plates were sub cultured for further identification (Obirikwurang *et al.,* 2012).

Colony counts

Colonies were counted on Nutrient agar using electric colony counter. A bacterial count of 10^5 per ml was considered significant for urinary tract infection (UTI) and counts of 10^2–10^4 per ml were considered as suspected bacteriuria while counts less than 10^2 per ml were considered as non-significant bacterial growth (Obirikwurang *et al.,* 2012).

Characterization and Identification of the Isolates

The growth on the mixed culture plates were sub cultured on Nutrient agar and incubated aerobically at 37°C for 24 hrs. Growths on the culture media were identified using the colony descriptions of the isolates, morphological characteristics and

biochemical reactions of the isolates (Obirikwurang *et al.*, 2012).

Preparation of the Test Organisms for Sensitivity Test

This was carried out using the method of Obirikwurang *et al.*(2012). The isolates were sub cultured on nutrient broth and incubated aerobically at 37°C for 24hrs. Broth cultures of the isolates were centrifuged at 3000 rpm for 10 minutes. The sediments were diluted with sterile phosphate buffer saline (PBS) and adjusted to the 10^8CFU/ml using McFarland matching standard (mixture of 0.6ml of 1% $Bacl_2.H_2O$ and 99.4ml of 1% conc. H_2SO_4) using spectrophotometer at 540nm.

Antibiotic Sensitivity Testing

The disc diffusion method was used to carry out the antibiotic sensitivity testing. The test organism was seeded on Mueller Hinton agar using pour plate method, and allowed to solidify. A sterile forceps was used to place the antibiotic sensitivity disc on the surface of the medium. The set-up was incubated aerobically at 37°C for 24 hrs. The inhibition zone diameters were measured using meter rule after 24 hrs incubation and recorded.

Results and Discussion

Out of the hundred (100) urine samples collected from the students staying in female hostel of Chukwuemeka Odumegwu Ojukwu University, Uli Campus, 29 samples were significant for UTI (10^5 CFU/ml), 34 samples were suspected for UTI (10^2–10^4 CU/ml) while 37 samples were not significant for UTI (below 10^2 CFU/ml) (Tables 1, 2 and 3).

Bacteriological analysis of the urine samples based on the age of the students is shown in Table 1. Samples collected from students between 21–24 years were most significant for UTI(37.88%) while samples from students above 25 years were the least.

Table 2 shows the bacteriological analysis of the urine samples based on the marital status of the students. The result reveals that out of 94 samples collected from single female students, 29 samples were significant for UTI whereas no sample was significant among the six (6) samples collected from married female students. Also, only two (2) married students out of six (6) married students were suspected to have UTI.

Bacteriological analysis of the urine samples based on the students' field of study among the female students is shown in table 3. The result showed that the urine samples collected from non medical students were most significant for UTI(48.48%) followed by samples from medical related students, while samples from medical students showed the least (14.29%).

The frequency of bacteria isolated from the urine samples is presented in Table 4. The result shows that *Escherichia coli* (56.38%) was the predominant organism isolated from the urine samples followed by *S. saprophyticus, S. aureus, K. pneumoniae* while *P. mirabilis* (4.26%) was the least.

Tables 5 and 6 respectively show the susceptibility study of Gram positive and Gram negative bacteria isolated from the studied urine samples against conventional antibiotics. Ciprofloxacin showed the most pronounced activity against the Gram positive and Gram negative bacteria studied with inhibition zone diameters ranging between 18-19.5mm and 22.5-26mm respectively *Escherichia coli* was resistant to nalidixic acid and ampicilin while *Proteus*

mirabilis was resistant only to ampicilin. *Klebsiella pneumoniae* was resistant to streptomycin, septrin and nalidixic acid. *Staphylococcus aureus* and *Staphylococcus saprophyticus* were resistant to bacitracin, azithromycin, lerofloxacin, amikacin and vancomycin.

The present study revealed that out of the one hundred (100) urine samples collected from the female students, 29(29%) samples were significant for UTI (10^5 CFU/ml), 34(34%) samples were suspected for UTI (10^2–10^4 CU/ml) and 37(37%) samples were not significant for UTI (below 10^2 CFU/ml). The occurrence of UTI among the students could be attributed to the poor sanitary conditions of the environment due to over congestion of the hostel. Similar conclusions were drawn by different researchers (Bloomberg *et al.,* 2005; Obirikwurang *et al.,* 2012). The study showed that urine samples collected from students between 21–24 years were most significant for UTI. The highest cases of UTI among students between 21–24 years could be attributed to the fact that most females in this group are young highly sexually active females. Some engage in sexual intercourse more than required, changes of sexual partners and use of spermicides (Bint and Hill, 1994). It has also been documented that in young sexually active women, sexual activity is the cause of 75–90% of bladder infections, with the risk of infection related to the frequency of sex (Nicolle, 2008). Also, biological and physical factors could contribute to the increase in cases of UTI such as having shorter distance between the urethra and anus.

The absence of significant UTI among the samples collected from married females in the hostel could be attributed to the low rate of sexual intercourse, adherence to single partner and high degree of hygienic condition. Several studies had shown that good sanitary condition and decent sexual pattern reduces occurrence of UTI (Boekitwetan *et al.,* 2012). There is also a possible link between the prevalence of UTI among students and the level of personal hygiene or the state of toilet facilities in the hostels (Ojo and Anibijuwon, 2010).

The present study highlighted that urine samples collected from students from medical fields were least significant to UTI. This could be due to these students being exposed and thoroughly taught the risk factors associated with the cases of UTI, and they decided to put them into practice. Bloomberg *et al.* (2012) highlighted that education on the risk factors associated with UTI, is one of the major ways of reducing cases of UTI.

The study revealed the presence of *Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli, Proteus mirabilis* and *Klebsiella pneumoniae.* Most of these organisms have been isolated by many researchers (Bint and Hill, 1994; Bloomberg *et al.,* 2005; Boekitwetan *et al.,* 2009; Obirikorang *et al.,* 2012; Geofrey *et al*.,2013; Poonam and Ulka, 2013). The highest occurrence of *E. coli* in the urine samples correlated with the results of Poonam and Ulka (2013). Similar conclusions were drawn by other researchers (Nadia *et al*., 2004; Bloomberg *et al.,* 2005; Ojo and Anibijuwon, 2010; Boekitwetan *et al.,* 2012; Geofrey *et al*.,2013;). From this study, *Staphylococcus saprophyticus* was the second most predominant (20.21%) microorganism isolated. This does not agree with the work of Nadia *et al*.(2004) who found out that among bacteria isolated from urine samples of urinary tract infection patients, *Staphylococcus saprophyticus* was the least (1.5%).

Table.1 Bacteriological Analysis of the Urine Samples According to the Age of the Students

Age (in years)	No. of samples	Significant (%)	Suspected (%)	Not significant (%)
Below 20	44	11 (25.00)	15 (34.09)	18 (40.91)
21–24	37	14 (37.88)	11 (29.73)	12 (32.43)
25 and above	19	4 (21.05)	8 (42.11)	7 (36.84)
Total	100	29 (29.00)	34 (34.00)	37 (37.00)

Table.2 Bacteriological Analysis of the Urine Samples According to the Marital Status of the Students

Marital status	No. of samples	Significant (%)	Suspected (%)	Not significant (%)
Single	94	29 (30.85)	32 (34.04)	33 (35.11)
Married	6	0 (0.00)	2 (33.33)	4 (66.67)
Total	100	29 (29.00)	34 (34.00)	37 (37.00)

Table .3 Bacteriological Analysis of the Urine Samples based on the Students field of Study

Field of study	No. of sample	Significant (%)	Suspected (%)	Not significant (%)
MS	21	3 (14.29)	7 (33.33)	11 (52.38)
MRS	46	10 (21.74)	16 (34.78)	20 (43.48)
NMS	33	16 (48.48)	11 (33.33)	6 (18.18)
Total	100	29 (29.00)	34 (34.00)	37 (37.00)

MS = Medical Students, MRS = Medical Related Students, NMS = Non Medical Students

Table.4 Frequency of Isolation of the Bacteria from the Urine Samples

Isolate	Frequency	Percentage (%)
Staphylococcus aureus	11	11.70
Staphylococcus saprophyticus	19	20.21
Escherichia coli	53	56.38
Proteus mirabilis	4	4.26
Klebsiella pneumoniae	7	7.45
Total	94	100

Table.5 Susceptibility Study of Gram Negative Isolates Against the Tested Antibiotics

Antibiotics	*E. coli*	*P. mirabilis*	*K. pneumoniae*
CPX	24.50	26.00	22.50
CN	20.00	23.50	19.50
OFX	14.00	21.00	17.00
PN	□	□	11.50
PEF	15.00	17.50	17.50
CEP	18.00	14.50	12.00
S	19.00	18.00	□
SXT	11.00	16.50	□
NA	□	11.50	□
AU	22.50	20.50	18.50

OFX = Tarivid, NA = Nalidixic acid, AU = Augmentin, S = Streptomycin, CEP = Ceporex, CN = Gentamicin, PEF = Reflacine, CPX = Ciprofloxacin, PN = Ampicilin, SXT = Septrin,

□ = No zone of inhibition

Table.6 Susceptibility Study of Gram Positive Isolates against the Tested Antibiotics

Antibiotics	Inhibition Zone Diameter (mm)	
	S. aureus	*S. saprophyticus*
B	□	□
AZM	□	□
CPX	18.00	19.50
LEV	□	□
CN	13.00	15.50
PN	12.00	13.50
AN	□	□
CRO	17.50	18.00
CTX	14.00	16.00
VA	□	□

B = Bacitracin, LEV = Levofloxacin, AN = Amikacin,VA = Vancomycin, AZM =Azithromycin, CN = Gentamicin,CTX = Cefotaxime,CPX = Ciprofloxacin, PN = Ampicilin,CRO= Ceftriaxone, □ = No zone of inhibition

Ciprofloxacin was proved to be most effective against the Gram positive and Gram negative bacteria studied in this work. This agrees with the findings of Geofrey *et al*.(2013) in which the isolated Gram positive and Gram negative bacteria all showed 100% sensitivity towards Ciprofloxacin.This could be attributed to the wider spectrum of activity of this antibiotic (Arora and Arora, 2008). The resistance exhibited by the isolates against some of the conventional antibiotics could be attributed to the ability of these organisms to acquire some mechanisms which might be genetic or acquired features, which allow the organism to resist the actions of the antibiotics. Similar conclusions were drawn by other researchers (Delzell and Leferre, 2000; Boekitwetan *et al.,* 2012).

The present study has shown that the urine samples collected from female students staying in female hostel of Chukwuemeka Odumegwu Ojukwu University, Uli campus showed significant UTI, of which the cases were most among single females, students that studied non medical related courses and students between the ages of 20–24 years. *Escherichia coli* was the most predominant organism isolated from the urine samples. The conventional antibiotics showed pronounced activities against the isolates, of which ciprofloxacin recorded the highest activity against Gram positive and Gram negative bacteria studied. Therefore, a periodic testing for UTI is advocated and those found to be infected need to be treated with antibiotics like ciprofloxacin to avoid complications.

Reference

Arora, D.R. and Arora, B. 2008. Text Book of Microbiology, third edition. CBS Publishers, New Delhi, India, pp. 437-441.

Bint, A.J. and Hill, D. 1994. Bacteriuria of Pregnancy - an update on significance, diagnosis and management. *J. Antimicrob. Chemother*. 33(2): 93-97.

Bloomberg, B., Oslen, B., Hinderaker, S., Langeland, N., Gasheka, P., Jureen, R., Kvale, G. and Midtvedt, T. 2005. Antimicrobial resistance in urinary bacterial isolates from pregnant women in rural Tanzania: Implications for public

health. *Scand. J. Infect. Dis.* 37(3): 262-268.

Boekitwetan, P.P., Suryawidjaja, J.E., Aidilfit, M. and Lesmana, M. (2009). Multimicronutrient supplementation and asymptomatic urinary tract infections in elderly. *Uni. Med.*28(3): 25-33.

Colgan, R. and Williams, M. 2011. Diagnosis and treatment of acute uncomplicated cystitis. *Am. Fam. Phy*. 84 (7): 771–776.

Delzell, J.E. and Lefevre, M.L. 2000. Urinary tract infections during pregnancy. *Am. Fam. Phy*. 61(3): 713-721.

Dielubanza, E.J. and Schaeffer, A.J. 2011. Urinary tract infections in women. *The Med. Clin. North Amer*. 95(1): 27–41.

Geofrey, A.O., Scolastica, C.K., Joan, C.C., Ongechi, D.R., Benard, M.M., Godfrey, O.M., Eliakim, M.M. and Isabella, J.K. 2013. Isolation, identification and characterization of urinary tract infectious bacteria and the effect of different antibiotics. *J. Nat. Sci. Res*. 3(6): 150-159.

Goossens, H. and Sprenger, M.J.W. 1998. Community acquired infections and bacterial reistance. *Biol. Med. J*. 317:654-657.

Lane, D.R. and Takhar, S.S.2011. Diagnosis and management of urinary tract infection and pyelonephritis. *Emerg. Med. Clin. North Am*. 29(3): 539–52.

Nadia,G., Talat, Y.M. and Samia, A. 2000. Isolation, identification and antibiotic resistance profile of indigenous bacterial isolates from urinary tract infection patients. *Pak. J. Biol. Sc*. 7(12):2051-2054.

Nicolle, L.E. (2008). Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. *Urol. Clin. North Am.* 35(1): 1-12.

Obirikwurang, C., Quaye, L., Bio, F.Y. and Amidu, N. 2012. Asymptomatic bacteriuria among pregnant women attending antenatal clinic at the University Hospital, Kumasi, Ghana. *J. Med. and Biomed. Sc.* 1(1): 38-44.

Ojo, O.O. and Anibijuwon, I.I. 2010. Urinary tract infections among female students residing in the campus of the University of Ado Ekiti, Nigeria. *Afr. J. Microbiol. Res*. 4(12):1195-1198.

Pallett, A. and Hand, K. 2010. Complicated urinary tract infections: practical solutions for the treatment of multiresistant Gram-negative bacteria. *J. Antimicrob. Chemother*. 65 (Suppl 3):25–33.

Poonam, U.S. and Ulka, B. 2013. Isolation and identification of bacteria causing urinary tract.infections in pregnant women in Vidarbha and their drug susceptibility pattern in them. *Inter.J. Curr. Microbiol. Appl.* Sc. 2(4):97-103.

Rubin, R.H., Shapiro, E.D., Andriole, V.T., Davis, R.J. and Stamm, W.E. 1992. Evaluation of new anti-infective drugs for the treatment of urinary tract infection. *Clin. Infect. Dis.* 15(1): 216–227.

Salvatore, S., Salvatore, S., Cattoni, E., Siesto, G., Serati, M., Sorice, P. and Torella, M. 2011.

Urinary tract infections in women. *Eur. J. Obstet. Gynecol. Reprod. Biol*. 156 (2): 131–136.

Shepherd, A.K. and Pottinger, P.S. 2013. Management of urinary tract infections in the era of increasing antimicrobial resistance. *Med. Clin. North Am*. 97(4): 737–57.