Magnitude and characteristics of urinary tract infection among Caritas university students: A Pilot Study

ABSTRACT

Fifty students residing in the campus of Caritas University Amorji-nike Enugu were examined for significant bacteriuria indicative of urinary tract infections (UTI) using cultural methods. Analysis of their clean cached mid-stream urine samples revealed that 24 (48%) students (both males and females) have significant bacteria, 16 (32%) indicated insignificant bacteria while 10 (20%) were negative because they showed no growth. The twenty-four (24) bacterial isolates were tentatively characterized into five genera namely *Escherichia coli* (33.33%), *Klebsiella pneumomia* (29.17%), *Proteus mirabilis* (20.83%), *Staphylococcus aureus* (12.5%), *Pseudomonas saureoginosa* (4.17%). Factors responsible for frequent cases of UTI among diagnosed women include shortness of urethra among the females, lack of personal hygiene, sexual intercourse, socio-economic challenges in various homes and others. Invitro antibiotic susceptibility test revealed that the gram negative bacteria were sensitive to quinolones (Ofloxacin, Ciprofloxacin, Pefloxacin), while the gram positive isolates were sensitive to erythromycin, levofloxacin, Gentamicin, Clindamycin and quinolones (Ofloxacin and Ciprofloxacin). Nitrofurantoin, Ampicillin, Ampiclox and Augmentine used in this study, were poorly effective, therefore, the use of the quinolones which includes Ofloxacin, Ciprofloxacin and Pefloxacin is highly recommended for UTI cases

KEYWORD: UTI, urethra, gram negative bacteria , Ofloxacin, Ciprofloxacin , Pefloxacin

Introduction

Urinary tract infection (UTI) is an infection in any part of the urinary system – kidneys, ureters, bladder and urethra [1]. UTI is caused by pathogenic invasion of the urinary tract which leads to an inflammatory response of the urothelium. Worldwide, about 152 million people are diagnosed with urinary tract infections each year with morbidity of about 196,500 [2]. In developing countries like Nigeria, the rate of UTIs is high especially among women [3]. To distinguish between infected and contaminated urine specimen from asymptomatic patients, significant bacteriuria has been used. Patients without symptoms whose bacterial counts in their urine is greater than 10^4/ml of the urine specimen do not have UTI. This was explained by Mehvish and Betty [4]. Bacteriuria is defined as the presence of $>10^5$ colonies of a single pathogen per milliliter of urine. Urinary tract infection is more common in females than males as a result of opening of the urethra near the anus and shortness of urethra in females [5]. According to Akobi *et al* [6], urine of females has more suitable pH and osmotic pressure for the growth of *Escherichia coli* than urine from males.

The clinical manifestations of UTI depend on the portion of the urinary tract involved, the etiologic organism(s), the severity of the

infection and the patient's ability to mount an immune response to it [7]. Notable clinical symptoms of urinary tract infections include lower abdominal pain, urgency and frequency of micturition, dysuria, itching, pyuria, the formation of blisters and ulcers in genital areas. Other signs and symptoms may include fever, chills, dysuria, urinary urgency, frequency and cloudy or malodorous urine. Infections are almost always ascending in origin and caused by bacteria in the periurethral flora and the distal urethra [5].

UTIs are commonly caused by bacteria such as *Escherichia coli*, *Klebsiella sp*, *Proteus*, *Enterococcus faecalis*, and *Enterobacter*, *Pseudomonas aureuginosa*, *Staphylococcus aureus*. These bacteria inhabit the distal gastrointestinal (GIT) and colonize the perineal area. *E. Coli* usually causes a child's first infection [8] but other gram-negative bacilli and *Enterococci* may also cause infection. *Staphylococcal* infections, especially those due to *Staphylococcus saprophyticus* [9] are common causes of urinary tract infection among female adolescents. Group B *Streptococcus* is usually reported with increased rates in patients with urological disorders and following repetitive course of antibiotic treatment. The prevalence and the degree of occurrence of one or two of these organisms are dependent on the environment [1]. High sexual activities, poor hygiene, low economic status, are some risk factors that predispose adolescent and adult women with urinary tract infections [10]. Studies have shown that patients with UTI have only single bacterial species while the presence of two or more growths in UTI can be due to contamination [10].

According to Azubike et *al(*Azubike and Nkeaniginieme, 1999), The commonest mode of infection is the ascending route, through which organisms of the bowel flora contaminated the urethra, ascend to the bladder and migrate to the kidney or prostrate. Knowledge about the type of pathogens responsible for UTIs and their susceptibility patterns may help clinicians to choose the right empirical treatment [11].

UTI occurrence is widely related to social class, age of patients, birth rank or size of family. UTI affects people in varying incidences depending on age group and gender. Most times boys are at greater risk before the age of 3 months but girls become at greater risk thereafter. Studies have shown that 3% of pre pubertal girls and 1% of prepubertal boys are diagnosed with a UTI. In male infants, circumcision is associated with a decreased rate of UTI [12,13]. When all age groups are combined, women are at greater risk than men of developing a UTI [14][1]. For older men, the case is different due to increasing prostatic hypertrophy which may affect urine flow thereby increasing the risk of developing UTI. When UTI is present in older people, it is asymptomatic [15]. 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 [16]. Women are also prone to UTIs due to the shortness of the urethra and its closeness to the anus [17].

Therefore, this study aimed to determine the prevalence of urinary tract infection among

the study residing in the campus of Caritas University, Amorji-Nike Emene Enugu, to find out the causative organisms and find out suitable antibiotics capable of treating the infections.

Materials and Methods

Study area

The study was carried out among the undergraduates at Caritas University, Amorji-Nike, Enugu. A total of 50 subjects were studied. Urine samples were examined for significant bacteriuria.

Sample collection

Clean cached mid-stream early morning urine of 10-20mls was collected by the patients using sterile boric acid bottle. In the clean-catched mid-stream method, the first urine voided was not collected because it could be contaminated with those transient microorganisms normally found in the lower portion of the urethra [18].

Only the mid-stream portion was collected since it most likely would contain microorganisms found in the urinary tracts.

Sample Processing:

Method of Analysis of Urine Samples

Urine test covered urinalysis and MCS (Microscopy, culture and sensitivity)

Urinalysis

Microscopic examination which involved the assessment of physical properties of urine such as volume, colour, appearance, odour, foam and specific gravity were performed on each sample.

This helped investigate UTI and also for the detection of abnormal chemical substance in the urine, such as pH, protein, glucose, nitrite, leucocyte esterase was performed [5]. Dry reagents strips are commercially available for chemicals examination of urine. The combi-9 strip was dipped in urine, chemicals come in contact with the specific substance in the urine, thereby producing reaction, which is denoted by colour changed.

Microscopic Examination Of Urine Samples (Wet Preparation)

About 10ml of well-mixed urine were aseptically transferred to test tube and labeled. They were centrifuged at 3000rpm for 5mins. The supernatant was discarded and the deposit was remixed by tapping the bottom of the tube. One drop was transferred to a clean slide and covered with slip, then examined microscopically with the use of x10 and x40 objectives. These were done to detect and estimate pus cells, red cells, yeast cells, bacteria and casts in the urine.

Culturing of Urine Samples

Urine sample was cultured on MacConkey agar, on Cysteine Lactose Electrolyte Deficient (CLED) and Blood agar. The agar was prepared and sterilized based on the manufacturer's instruction using the autoclave. After sterilizing, the media was allowed to solidify and at temperature of 50^0C-55^0C, it was dispensed into sterile Petri dish. The inoculation was carried out and the media incubated for about 24hours

at 37^0C. The organism grown was sub-cultured so as to isolate pure cultures of organisms. Isolated bacterial species were characterized by Gram stain followed by microscopic examination, motility and biochemical tests and bacteria identification was based on standard culture and biochemical characteristics of the isolates as described by Omer and Fadil [19].

Antibiotics sensitivity test on isolates

Paper disc Diffusion Method

Sensitivity disc was placed on Mueller hintonagar inoculated with test organism and incubated at 37^0c overnight. The antibiotic diffused into the surrounding medium. The growths of organisms inhibited up to a distance of 10mm in diameter from the disc were considered as sensitive to the disc. While those that did not have inhibition zone up to that were considered as resistant to the disc. However, consideration was given to those antibiotics that have very low diffusing rate i.e. those with high molecular weights.

Results

Out of 50 urine samples collected from both males and females, 24 (48%) had significant bacteria count of more than 10^5CFU/ml, while 16 students (32%) have insignificant bacteria count ($<10^5$ CFU/ml). The other 10(20%) students were regarded as negative for bacteria ($<10^3$CFU/ml).

Table 1 shows the characteristics appearance of the bacterial isolates from the urine specimens under the microscope after staining and their biochemical tests. Out of 50 samples tested, 24(48%) were infected. In male students in 100level, 1(25%) was infected out of 4 samples. In male students in 200level, 2(40%) were infected out of 5 samples. In male students in 300 level, 4 (57.14%) were infected out of 7 samples while in male students in 400level, 6 (66.67%) infected out of 9 samples tested. In female students in 100 level, 2(50%) were infected out of 4 samples. In female students in 200 level, 4 (66.67%) were infected out of 6 samples while in female students in 300level, 3 (42.86%) infected out of 7 samples tested. In female students in 400level, 6 (75%) infected out of 8 samples tested. This is shown in table 2.

Table 3 shows the prevalence of UTI in relation to ages of female and male students. Results indicate that the high percentage of organisms were isolated from them within the ages of 18-20 years and 21-23 years comparatively, however, they were more cases in those within the age bracket of 21-23 than those of within the other age brackets.

The percentage occurrence of bacteria isolated in both is presented in Table 4. Out of the 50 examined 24 had significant bacteriuria. The bacteria isolated were *Pseudomonas aeruginosa*1(4.17%), *Staphylococcus aureus* 3(12.5%), *Escherichia coli* 8(33.33%), *Klebsiella pneumonia*7(29.17%) and *Proteus mirabilis*5(20.83%).

TABLE 1a: Morphological characteristics of Bacterial Isolates.

ISOLATE CODE	CULTURAL CHARACTERISTICS
1	Round creamy raised with smooth edges
2	Round creamy raised smooth edges
3	Round gray spreading isolate with a shiny surface
4	Round cream raised and smooth edges
5	Round gray with a shiny surface
6	Round cream raised and shiny surface
7	Irregular cream raised and shiny surface
8	Irregular creamy rough edges that are slightly raised
9	Round creamy smooth edges
10	Round creamy smooth edges
11	Roudgray shiny surface, with smooth edges
12	Round gray shiny surface with smooth edges
13	Round creamy smooth edges and raised
14	Irregular creamy rough edges that are slightly raised
15	Round with smooth edges and slightly raised
16	Irregular yellowish colonies and slightly raised
17	Creamy flat with rough edges
18	Round yellowish colonies and slightly raised
19	Round yellowish colonies and slightly raised
20	Irregular creamy rough edges that are slightly raised
21	Irregular gray shiny surface with smooth edges
22	Round smooth edges, creamy, raised
23	Shiny, creamy, round with smooth edges
24	Irregular, flat, creamy and rough edges

TABLE 1b: Biochemical Tests of Isolates.

Isolates codes	Gram staining	Coagulase	Csatalase	Oxidase	Indole	Cellular morphology	Most probable isolates
1	-ve	-ve	+ve	-ve	+ve	Rods	*Escherichia coli*
2	-ve	-ve	+ve	-ve	-ve	Rods	*Klebsiella pneumonia*

3	-ve	-ve	+ve	-ve	-ve	Rods in chains	*Klebsiella pneumonia*
4	-ve	-ve	+ve	-ve	-ve	Rods in chains	*Proteus mirabilis*
5	+ve	+ve	+ve	-ve	-ve	Cocci in clusters	*Staphylococcus aureus*
6	-ve	-ve	+ve	-ve	+ve	Rods	*Escherichia coli*
7	-ve	-ve	+ve	-ve	-ve	Rods in chains	*Proteus mirabilis*
8	-ve	-ve	+ve	-ve	+ve	Rods	*Escherichia coli*
9	-ve	-ve	+ve	+ve	-ve	Rods	*Pseudomonas aeruginosa*
10	-ve	-ve	+ve	-ve	-ve	Rods	*Klebsiella pneumonia*
11	-ve	-ve	+ve	-ve	-ve	Rods in chains	*Proteus mirabilis*
12	-ve	-ve	+ve	-ve	+ve	Rods	*Escherichia coli*
13	-ve	-ve	+ve	-ve	-ve	Rods in chains	*Proteus mirabilis*
14	-ve	-ve	+ve	-ve	-ve	Rods	*Klebsiella pneumonia*
15	-ve	-ve	+ve	-ve	+ve	Rods	*Escherichia coli*
16	-ve	-ve	+ve	-ve	-ve	Rods	*Klebsiella pneumonia*
17	-ve	-ve	+ve	-ve	+ve	Rods	*Escherichia coli*
18	-ve	-ve	+ve	-ve	-ve	Rods	*Klebsiella pneumonia*
19	+ve	+ve	+ve	-ve	-ve	Cocci in clusters	*Staphylococcus aureus*
20	-ve	-ve	+ve	-ve	+ve	Rods	*Escherichia coli*
21	-ve	-ve	+ve	-ve	-ve	Rods	*Klebsiella pneumonia*
22	-ve	-ve	+ve	-ve	+ve	Rods	*Escherichia coli*
23	-ve	-ve	+ve	+ve	-ve	Rods in chains	*Proteus mirabilis*
24	+ve	+ve	+ve	-ve	-ve	Cocci in clusters	*Staphylococcus aureus*

KEY; -ve=(gram negative)+ve= (gram positive)

TABLE 2: Prevalence of UTI in relation to levels of Students (Male and Female)

Levels of students	Numbers of samples collected	Numbers of students infected

100	8 (4 male, 4 female)	1(25%) and 2(50%)
200	11 (5 male, 6 female)	2(40%) and 4(66.67%)
300	14 (7 male, 7 female)	4(57.14%)and 3(42.86%)
400	17 (9 male, 8 female)	6(66.67%)and 6(75%)
TOTAL	50	24 (48%)

TABLE 3: Prevalence of UTI in relation to Age (Male and Female)

Age Groups (Years)	Male and Female Examined	Male and female positive
15-17	10	3(30%)
18-20	14	7(50%)
21-23	17	10(58.82%)
Total	50	24(48%)

TABLE 4: Prevalence of Isolates

Isolate	**Number**	**Percentage number %**
E. coli	8	33.33

Klebsiella	7	29.17
Proteus mirabilis	5	20.83
Staphylococcus aureus	3	12.5
Pseudomonas	1	4.17
Total	**24**	**100.00**

Table 5a: Antimicrobial Sensitivity Reaction of Drugs on the Isolates

(Zone Of Inhibition in mm for Gram Negative)

TEST ORGANISMS	N 30 Mg	CT 30 µg	CIP 10 µg	GN 30 µg	OF 10 µg	AU 30 µg	PF 30 µg	CM 30 µg	C 10 µg	AM 30 Mg
E.coli	12	14	20	14	20	0	20	20	0	0
Klebsiella pneumonia	0	0	14	14	18	0	18	14	0	0
Pseudomonas	0	0	18	0	18	0	18	0	0	0
Proteus mirabilis	0	14	20	18	20	0	20	0	11	0

Table 5b: Antimicrobial Sensitivity Reaction of Drugs on the Isolates

(Zone Of Inhibition in mm for Gram Positive Bacteria)

TEST ORGANISIM	E	CT	AP	CE	LV	NB	CIP	GN	OF	CD
Staphylococcus aureus	20	12	0	10	30	15	28	18	28	28

KEY:

GRAM NEGATIVE

N- Nitrofurantoin

CT- Cetriaxone

CIP- Ciprofloxacin

GN- Gentamicin

OF- Ofloxacin

AU- Augumentine

PF- Pefloxacin

CM- Clanthomycin

C- Chloramphenicol

AM- Ampicillin

GRAM POSITIVE

E- erythromycin

CT- cetriaxone

AP- ampiclox

LV-levofloxacin

NB- norfloxine

CIP-ciprofloxacin

GN-gentamicin

OF-ofloxacin

CD-clindamycin

Discussion

Mid-stream catch technique has been used to ensure those normal floras being flushed before the sample for analysis was obtained [5]. Frequent voiding is necessary to flush out invading bacteria from the walls of urethra [5,20].

Fifty (50) urine specimens were examined for the presence of bacteria or otherwise. 24(48%) were found positive for UTI; having greater than 10^5CFU/ml of bacteria of clean voided, mid-stream urine. However, 16(32%) specimen have insignificant or doubtful bacteria having less than 10^5CFU/ml and 10(20%) other specimen showed a negative result. This is not unexpected in a normal healthy individual or could be due to indiscriminate consumption of antibiotics by students since some of them confessed using non prescribed antibiotics whenever difficulty urination is noticed. The insignificant or doubtful bacteria could also be due to contamination during specimen collection. Although the students were educated on how to collect the specimen aseptically, maximum efficiency cannot be guaranteed since most students are not sterility conscious. From the private

interviews conducted with students, it was observed that most of the UTI cases were asymptomatic except very few cases (most of the students do not experience lower abdominal pain, difficulty in urination and other characteristics symptoms of UTI, but have significant bacteria).

The study implicated five microorganisms as possible etiological agents of UTI cases observed. These organisms include; *Escherichia coli* (33.33%), *pseudomonas aeruginosa* (4.17%), *staphylococcus aureus* (12.5%), *klebsiella pneumonia* (29.17%), *proteus mirabilis* (20.83%) as the common causative agents of UTI this result compares favorably with results by Smith, (2003)[21]; This finding is similar to other report which indicates that gram negative bacterium, particularly *Escherichia coli*, is the commonest pathogen isolated in students with UTI [24- 26]. This is in line with the work of Doud *et.al* [27] also Onwujiekwe et al discovered the dominance of *Staphylococcus aureus* followed by *Escherichia coli in* the urine samples examined [23]. The reason for the high prevalence of *Escherichia coli* can also be attributed to the suitable pH and osmotic pressure provided by the female urine [6]. However, other authors have been stated that Escherichia coli bacteria are a normal inhabitant of the GIT including the rectum and anal canal. Therefore, any sexual manipulation of the anal region instead of the normal vaginal intercourse could lead to more UTIs in males than females [28-30].

On the course of the study the higher prevalence of *Escherichia coli* (33.33%) may be due to fecal contamination, the predilection of the form toilets and the shortness of the female urethra [16]. This prevalence however, is also reported in the earlier works by smith *et al.,* (2003)[21], where they found out that *Escherichia coli* accounts for 32% of UTI cases. *Proteus mirabilis* with 20.83%prevalence has a significant association with UTI. Its active motility and swarming ability can in comparison with other organisms' transverse easily through the urethra.

There is also possible link between the prevalence of UTI among students and the level of personal hygiene or the state of toilet facilities in the hostels. Most of the students examined complained of poor toilet management that is no adequate supply of water and disinfectant to clean and flush the toilet regularly. When toilets are dirty, there is an accumulation of urine sediments forming a thick scum. In this case students can be infected when they make use if the toilet. Sexual activities are another factor that predisposes people to UTI. *Staphylococcus aureus* for example, which is one of skin normal flora might stay on the skin and get transmitted during sexual intercourse.

Table 3 shows the prevalence of UTI in relation to the age of the male and female students. It was observed that majority of the positive cases fall between the age 21-23 years. 58.82% of the total sample population falls within this bracket inferring that most of the students within this age bracket are sexually active. 50% of the population is within 18-20 years, which infers also that those within this age bracket are also sexually active while 40% are above

26 years. Sexually activities enhance better transmission of UTI especially in female, who have higher prevalence of UTI than males.

The drug of choice shown by the sensitivity test for *E. coli* includes the following in their order of zone of inhibition, Nitrofurantoin (12mm), Ceftriaxone and Gentamicin (14mm) for each, Ciprofloxacin, Ofloxacin, Pefloxacin and Clanthomycin (20mm) for each, while the organism is resistant to ampicillin, Augumentine, Chloramphenicol. The drugs used for sensitivity test for *Klebsiella pneumonia* includes Ofloxacin and Pefloxacin (18mm) for each, ciprofloxacin, gentamicin, Clanthomycin (14mm) for each, while the organisms were resistant to Nitrofurantoin, Ceftriaxone, Augumentin and Chloramphenicol. The drugs used for the sensitivity test for *Proteus mirabilis*includes the following Chloramphenicol (11mm), Cetriaxone (14mm), Gentamicin, and Clanthomycin (18mm) for each, Ciprofloxacin, Ofloxacin, and Pefloxacin, (20mm) for each, while resistant to Nitrofurantoin, Augumentine, and Ampicillin. Drugs used for the sensitivity test for *Pseudomonas aureoginosa* includes the following in their zone of inhibitions, Ciprofloxacin, Ofloxacin, and Peloxacin (18mm) for each, while the organism is resistant to Nitrofurantoin, Augumentine, Ampicillin, Cetriaxone, Clanthomycin, Gentamicin, and chloramphenicol. The drugs used for the sensitivity test for *Staphylococcus aureus* include the following in their order of inhibition. Erythromycin and Gentamicin (18mm) for each, Cetriaxone (12mm), Cifixime (10mm), Levofloxacin (30mm), Norafloxacin (14mm), Ciprofloxacin and Clindanycin (28mm)for each and Ofloxacin (>28mm). The organism is resistant to Ampiclox. Therefore, from the results of the antimicrobial sensitivity test performed, it can be deduced that the most useful antibiotics in this study were quinolones (Ofloxacin, Ciprofloxacin and Pefloxacin), Erythromycin, Gentamicin, Levofloxacin and Clindamycin (in gram positives) as shown in table 5a and 5b, because they inhibit most commonly isolated UTI pathogens. This sensitivity test is very important because some of this bacteria species are resistant to some of these antibiotics. It is only through sensitivity test that one would know the drugs which the bacterium is susceptible to and also the drug that would be effective.

Conclusion

Escherichia coli are a predominant organism causing urinary tract infections in adolescent and adult women as a result of unprotected sex and high sexual activity at their age. *Staphylococcus aureus* are also frequently isolated organism that causes UTIs. Urinary tract infections occur mostly in females than their male counterparts. It has dangerous effect when left untreated. The prevalent isolates were susceptible to quinolones (Ofloxacin, Ciprofloxacin and Pefloxacin), Erythromycin, Gentamicin, Levofloxacin and Clindamycin (in gram positives). This study advocates early diagnosis of urinary tract infection, regular monitoring of drug resistant phenotype of UTI pathogens to improve public health treatment and reduce cases of infections with other complications caused as a result of urinary pathogens in

our society also Due to the high prevalence level of UTI cases in females, they should be enlightened on the menace of UTI by organizing public health programs on the factors promoting the occurrence of UTI such as sexual activities, personal hygiene on campus among others and the ways to mitigate or reduce the occurrence of such in them and this measures also applies to the male even though there is a low level of UTI cases among them.

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