

Incidence of Urinary Tract Infections, among Adolescent and Adult Women in Ogbete Coal Camp, Enugu

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Abstract This study was to determine the bacteria that are prevalent in Urinary tract infections and the sensitivity of organisms isolated from the urine of adolescent and adult women of 8- 52 years old in Ogbete Coal camp in Enugu metropolis, south east Nigeria. A total of 218 individuals, both in and out patients were investigated, 197 (90.3%) women were diagnosed for Urinary tract infections. Therefore, incidence of UTI were prevalent in the age groups of 13-17 and 18-22, with P value ($p=0.047$ and S.D of ± 5.6). The microorganisms implicated in infection were bacteria, fungi, yeast and protozoa. Among the bacteria, two were identified as Gram positive cocci i.e. *Staphylococcus aureus* and *Staphylococcus saprophyticus*, while Gram negative bacilli were *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas aeruginosa*. Yeast like fungus *Candida albican*, protozoan (*Trichomonas vaginalis*) was also identified. The isolated organism which was prevalent was *Staphylococcus aureus* (48.2%); followed by *Escherichia coli* (34.5%). Also, *Pseudomonas aeruginosa*, *Proteus* and *Klebsiella* were isolated. Factors responsible for frequent cases of Urinary tract infections among diagnosed women include shortness of urethra among the females, lack of personal hygiene, sexual intercourse, socio economic challenges in various homes and others. Sensitivity profiles revealed that Augumentin was highly susceptible to *Staphylococcus aureus* (65%) while *Escherichia coli* were highly resistant to commonly used drugs such as Ampicillin, and Cloxacillin while Gentamycin was the most active against bacteria isolates.

Keywords Urinary Tract Infection, Adolescent Women, Antimicrobial Sensitivity, *Staphylococcus aureus*, *Escherichia coli*

1. Introduction

Urinary tract infection (UTI) is an infection in any part of the urinary system – kidneys, ureters, bladder and

urethra [1]. Worldwide, about 152 million people are diagnosed with urinary tract infections each year with morbidity of about 196,500 (WHO; 2017 [2]). In developing countries like Nigeria, the rate of UTI's is high especially among women [3]. 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 [4]. According to Akobi *et al* [5], urine of females has more suitable pH and osmotic pressure for the growth of *Escherichia coli* than urine from males. Urinary tract infection is classified into six categories as uncomplicated infection, complicated infection, isolated infection, unresolved infection, re-infection, and relapse infection [6]. Notable clinical symptoms of urinary tract infections include lower abdominal pain, urgency and frequency of micturition, dysuria, itching, pyuria, formation of blisters and ulcers in genital areas. Bacteria are the main causes of urinary tract infections among individual worldwide [7]. Urinary tract infections are commonly caused by bacteria such as *Escherichia coli*, *Klebsiella sp*, *Proteus*, *Enterococcus faecalis*, and *Enterobacter*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. 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 (Omonigho *et al*, [1]). High sexual activities, poor hygiene, low economic status, are some risk factors that predispose adolescent and adult women with urinary tract infections [9]. According to Azubike *et al*, [10], 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 Urinary tract infections and their susceptibility patterns may help clinicians to choose the right empirical treatment [11]. Therefore, this study aims to determine the bacteria that are prevalent in urinary tract

infections in adolescent women and adult women, and also the antimicrobial susceptibility profiles of drugs used in the treatment of such infections.

2. Materials and Methods

Study Area

Ogbete Coal camp area of Enugu is located in Enugu North local government in the heart of Enugu. The area is densely populated as a result of its proximity to Ogbete main market, which is the largest market in Enugu state. Also the presence of an automobile market at the Ogbete Coal camp town contributed to the dense population of this area. Therefore, the people of this area are mainly traders, artisans and students. There is government owned institutions as well as hotels around the area.

Study Population

The research was a retrospective studies carried out between May 2016 and May 2017 and was conducted in the microbiology section at Ogbete Coal camp Health Centre for urinary tract infections issues. This got the approval of the ethical committee of the Health Centre. Verbal informed consent was obtained from all patients prior to specimen collection and the permission to that effect was obtained from the ethical committee of the health center. Their urine sample was obtained through a mid-stream catch technique.

Sample Collection

Two hundred and eighteen (218) mid-stream urine (MSU) samples were collected inside sterile bijou bottles from the patients. They were instructed on how to collect the samples. The samples were labelled and processed within 30 minutes to 1 hour of sample collection.

Ten ml of each well mixed urine sample was centrifuged at 2000g 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, cast, crystal, yeast-like cells and *Trichomonas vaginalis*. Pus cells $>_5$ per high power field were significant in an individual.

List of Materials and Reagents

Materials used in the study includes spatula, bijou bottles, stopper, masking tape, conical flask, autoclave, gas cylinder, Bunsen burner, incubator, refrigerator, electronic weighing balance, colony counter, marker, Petri dish, wire loops, slides, test tube glass, pipette, centrifuge, microscope. Nutrient agar, MacConkey agar, blood agar, distilled water, crystal violet, lugol iodine, acetone,

caramel, and antimicrobial sensitivity disc.

Sample Processing

Urine sample were cultured on MacConkey agar 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°C-55°C, it was dispensed into sterile Petri dish. The inoculation was carried out and the media incubated for about 24hours at 37°C. The organism grown was sub-cultured so as to isolate pure cultures of organisms. A count of $>.10^5$ CFU (Colony forming unit) was considered significant to indicate UTI. 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 [12] and Cheesbrough [4].

Antimicrobial Sensitivity Testing

The susceptibility testing was carried out using disc diffusion technique as recommended by National Committee for Clinical Laboratory Standards (NCCLS) [13]. The bacterial isolates and standards strains of *Escherichia coli* ATCC 25922, *Enterococcus faecalis* 29212, *Pseudomonas aeruginosa* ATCC 27853 were used for quality control. The antimicrobials used are: Ceftriaxone, Ampicillin, Cloxacillin, Augmentin, Streptomycin, Azythromycin, Gentamycin, Cefuroxime, Cotrimoxazole, Nitrofurantoin, Nalidixic Acid.

Statistical Analysis: This data was analysed using SPSS version 20.0.

Results

A total of 197 (90.3%) out 218 samples collected were confirmed positive with urinary infections. The age distribution of urinary tract infections in women are shown in table 1. Out of the nine age groups represented in the table, positive bacterial isolates was prevalent in individuals between 18 -22, and 13-17 years. Bacterial isolates was least in individual between the age group 47-52. The bacterial species implicated in the urinary tract infections are *Escherichia coli*, *Klebsiella* sp, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The frequency of occurrence of isolates is presented in table 2. *Staphylococcus aureus* was found to be the most prevalent organism among the isolates, with 48.2% occurrence. This was followed by *Escherichia coli* which accounted for 34.5 % of the isolates. *Staphylococcus saprophyticus* was least frequently isolated.

Table 3; showed varying antimicrobial susceptibility

pattern of isolates to the various antimicrobials used. Gentamycin was highly effective against most bacterial isolates. Ampicillin was virtually useless against all bacterial isolate. Table 5, showed the characteristics of bacteria isolates. Table 6, showed the microscopy findings of urine deposits.

Discussion

Mid-stream catch technique was used to ensure those normal floras were flushed before the sample for analysis was obtained [4]. Frequent voiding is necessary to flush out invading bacteria from the walls of urethra [4, 14].

The result of this study showed that among the population of 218 samples, 197 (90.3%) were diagnosed positive of bacterial infections. Therefore, incidence of UTI were prevalent in the age groups of 13-17 and 18-22, with P value ($p=0.047$ and S.D of ± 5.6) statistically not significant. The frequency of occurrence of isolates is presented in (Table 2). *Staphylococcus aureus* is a predominant pathogen which accounted for 48.2 % of the bacteria isolates that caused urinary tract infection in adolescent women; this may be attributed to high sexual activities among their age group [9]. *Escherichia coli* also had a significant number which accounted for 34.5% of the isolates that caused urinary tract infection in women. This is in line with the work of Doud *et al* [15]. 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 [5]. *Klebsiella sp* was responsible for 6.6% of the isolates. *Proteus* specie accounted for 4.0%; a Gram- negative bacterium and flora of the intestinal tract that causes infection when it makes its way from the intestine to urinary bladder and urethra. *Staphylococcus saprophyticus* was the least among the bacteria isolates with 1.5% occurrence. However, this result agrees with the findings of MMDT, that about 40% of the urinary tract infections are caused by Gram negative species [16].

This study (Table 1) reports high incidence of UTI's between age groups 18 and 22years (98.1%), and 13-17 years (96.1 %). The most common uropathogen isolated in this study was *Staphylococcus aureus*. This findings is similar to two reports in Benin City (urban settlement) [17], [18], and findings of Akinola *et al* [19]. These reports were on asymptomatic bacteriuria against the present study that deals on symptomatic bacteriuria. However, our findings are contrary to the findings of Bankole *et al* [9], and Akobi *et al* [5], who reported *Escherichia coli* as the predominant isolates.

The high incidence of urinary tract infection in women as earlier mentioned is as a result of shortness of urethra, high sexual activity and closeness of urethral opening to the anus [9]. Also, socio-economic factors, is also a major risk factor as mentioned earlier [10]. Some homes where some of the studied population lives, were highly polluted environment, with unsuitable toilet facilities thereby exposing such inhabitants to numerous pathogenic microorganisms.

Antibiotic abuse and practicing incomplete antibiotic dosage has highly promoted the dissemination of multi drug resistant bacteria [20]. *Staphylococcus aureus* was the highest uropathogen showed high susceptibility to Nitrofurantoin and least susceptible to Cloxacillin. *Staphylococcus saprophyticus* followed similar pattern of susceptibility with *Staphylococcus aureus*. The present study found the *Escherichia coli* and *klebsiella* species isolates to be highly resistant to the commonly used drugs such of the Penicillin group such as Ampicillin and Cloxacillin. Ahmed *et al* [6] reported that Amoxicillin, Ampicillin, and Sulfanomides are no longer the drug of choice for empirical treatment because of widespread emergence of resistance in 15-20% of *E.coli* in several areas of USA and other countries. However, the antimicrobial susceptibility profile indicates that Nitrofurantoin (69.1%) were the most active agents against *Escherichia coli*. Aminoglycosides, example (Gentamycin and Augmentin) are the next drugs that are most susceptible to the isolates by (58.8%) and (51.4%) respectively. This result is in agreement to the recommendations of Akobi *et al*, [5]. *Klebsiella pneumonia* was susceptible to Nitrofurantoin and Cefuroxime both by (76.6%), but was completely resistant to Ampicillin. *Proteus* was highly susceptible to Gentamycin and Ceftriaxone but was also highly resistant to Streptomycin, Ampicillin, and Nitrofurantoin. *Pseudomonas aeruginosa* was moderately susceptible to Gentamycin and Ceftriaxone. *Proteus sp* and *P.aeruginosa* isolates were resistant to most antimicrobials. Most bacterial isolates was least susceptible to Cotrimoxazole. This study is similar to the findings by Mezue *et al*, [21] at University of Nigeria found Cotrimoxazole to be virtually useless against uropathogen. The resistant of most of the isolates to the penicillin group could be as a result of long term use of these drugs over the years.

However, patients should be advised and encouraged to drink plenty of fluids (two to three liters per day) and to urinate frequently to help flush bacteria from the bladder. Avoiding multiple sexual partners will reduce the risk of both UTI's and sexually transmitted infections. Women are encouraged to avoid spermicidal contraceptive, diaphragms and vaginal douching, which may irritate the vagina and urethra; facilitate the entry and colonization of bacteria within the urinary tract. Meanwhile, some recent studies by Ahmed *et al* [6], [22], [23], [24], [25] have suggested alternative therapies other than antimicrobials in the treatment of RUTI'S. Some of the measures include the use of Cranberry juice and tablets, acupuncture, probiotics such as *Lactobacillus* found in fermented milk products; mainly yoghurt, as well as immunoprophylaxis. Nevertheless, more clinical studies need to be carried out to determine their role in RUTI prevention.

3. Conclusions

Staphylococcus aureus is 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. *Escherichia coli* are also frequently isolated organism that causes UTI's. Urinary tract infections occur mostly in females than their male counterparts. It has dangerous effect when left untreated. The prevalent isolates were susceptible to Augmentin,

Nitrofurantoin and Gentamycin. 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.

Table of Results

Table 1; showed prevalence of infections was most among women between the ages of 18-22 and 13 and 17 (98.1% and 96.1%) positive cases. Age group 47-52, had the least incidence rate with 5 (50%) positive cases.

Table 1. Incidence of UTI's in relation to Age among adolescent and adult women

Age interval	No Tested	% positive	% negative
8-12	19	11 (57.9)	8 (42.1)
13-17	51	49 (96.1)	2 (3.9)
18-22	53	52 (98.1)	1 (1.9)
23-27	11	8 (72.7)	7 (63.6)
28-32	37	35 (94.6)	2 (5.4)
33-36	14	10 (71.4)	4 (28.6)
37-41	8	6 (75)	2 (25)
42-46	14	11 (78.6)	3 (21.4)
47-51	10	5 (50)	5 (50)

Table 2. Prevalence of organisms isolated from the urine

Organism	Number of patients	Number of occurrence (%)
<i>Escherichia coli</i>	197	68 (34.5%)
<i>Klebsiella</i>	197	13 (6.6%)
<i>Staphylococcus saprophyticus</i>	197	3 (1.5%)
<i>Staphylococcus aureus</i>	197	95 (48.2%)
<i>Proteus</i>	197	8 (4.0%)
<i>P. aeruginosa</i>	197	11 (5.6%)

Staphylococcus aureus was most prevalent in the urine samples (48%), next was *Escherichia coli* 34% and the least prevalent was *Staphylococcus saprophyticus*.

Table 3; showed varying antimicrobial susceptibility pattern of isolates to the antimicrobials used.

Table 3. Susceptibility Profiles of Urinary Bacterial Isolates

ANTIBIOTICS	<i>E. coli</i> (N=68)	<i>K. pneumonia</i> (N=13)	<i>S. saprophyticus</i> (N=3)	<i>S. aureus</i> (N=95)	<i>P. mirabilis</i> (N=8)	<i>P. aeruginosa</i> (N=11)
CEFT	26 (38.2%)	4 (30.7%)	1 (33.3%)	19 (20%)	6 (75%)	0(0.0%)
AMP	2 (2.9%)	0 (0.0%)	0 (0.0%)	4 (4.2%)	0 (0.0%)	NA
CLOX	NA	NA	1 (33.3%)	14 (14.7)	NA	NA
AUG	35 (51.4%)	4 (30.7%)	2 (66.6%)	62 (65.2%)	0 (0.0%)	0 (0.0%)
STREPT	20 (29.4%)	6 (46.1%)	1 (33.3%)	20 (21.0%)	0 (0.0%)	4 (36.3%)
AZY	29 (42.6%)	4 (30.7%)	1 (33.3%)	32 (33.6%)	6 (75%)	0 (0.0%)
GENT	40 (58.8%)	4 (30.7%)	1 (33.3%)	29 (30.5%)	6 (75%)	4 (36.3%)
CEF	7 (10.2%)	10 (76.9%)	0 (0.0%)	18 (18.9%)	0 (0.0%)	0 (0.0%)
COT	9 (13%)	2 (15.3%)	1 (33.3%)	19 (20%)	4 (50%)	0 (0.0%)
NIT	47 (69.1%)	10 (76.9%)	2 (66.6%)	47 (49.4%)	0 (0.0%)	NA
NAL	27 (39.7%)	4 (30.7%)	0 (0.0%)	16 (16.8%)	0 (0.0%)	NA

Bacteria Isolates with Zone of inhibition = 16mm is sensitive while < 15mm is resistant to a particular antibiotics. CEFT= CEFTRIAZONE. AMP= AMPICILLIN. CLOX= CLOXACILLIN. AUG= AUGUMENTIN. STREPT=STREPTOMYCIN. AZY= AZYTHROMYCIN. GENT= GENTAMYCIN. CEF=CEFUROXIME. COT= COTRIMOXAZOLE. NIT= NITROFURANTOIN. NAL=NALIDIXIC ACID. N= TOTAL NUMBER.

A total of 42 samples were resistant to either one or two antimicrobials used in bacteria susceptibility testing. *Staphylococcus aureus*, the most frequently isolated bacteriuria showed high susceptibility to Augmentin (65.2%) and Nitrofurantoin (49.4%) and least susceptible to Ampicillin (4.2%). *Escherichia coli*, second frequently isolated bacteriuria was susceptible to Nitrofurantoin (69.1%) and Gentamycin (58.8%) but least susceptible to Ampicillin (2.9%). *Klebsiella sp* was susceptible to Ceftriaxone and Nitrofurantoin both by (76.9%), however was completely resistant to ampicillin by (100%).

Staphylococcus saprophyticus was susceptible to Augmentin and Nitrofurantoin both by (66.6%) and completely resistant to Ampicillin and Nalidixic acid both by (100%). *Proteus mirabilis* was susceptible Ceftriaxone, Azithromycin, and Gentamycin by (75.5%) but showed (100%) resistance to Ceftriaxone, Augmentin, Streptomycin, Cefuroxime, Nitrofurantoin and Nalidixic acid. *Pseudomonas aeruginosa* was moderately susceptible to Streptomycin and Gentamycin both by (36.6%) and resistant to other antimicrobials.

Table 4. Pattern of bacterial occurrence in age groups

Age group	<i>E. coli</i>	<i>klebsiella</i>	<i>S. aureus</i>	<i>proteus</i>	<i>p. aeruginosa</i>	<i>S. saprophyticus</i>
8-12	3	1	8	-	-	-
13-17	14	1	33	-	-	1
18-22	20	5	22	2	1	2
23-27	3	1	4	-	-	-
28-32	16	2	11	2	6	-
33-37	7	-	2	-	1	-
38-42	1	2	3	4	1	-
43-47	4	1	6	-	2	-
48-52	2	-	4	8	-	-

Staphylococcus aureus was found to be main urinary pathogen among children between 8-11 years. It was observed that *Escherichia coli* and *Staphylococcus aureus* were implicated in infections in adolescent between 18-22 years as well as in women between 42-46, and 47-51

Table 5. Identification of characteristics of bacterial isolates

Catalase	Coagulase	Oxidase	Urease	Indole	Glucose	Sucrose	Lactose	Organism
+	-	-	-	+	A	A	AG	<i>Escherichia coli</i>
+	ND	-	+	-	AG	A	AG	<i>Klebsiella sp.</i>
+	+	-	-	ND	A	A	A	<i>S. aureus</i>
-	ND	-	+	+	-	A	ND	<i>Proteus</i>
ND	ND	+	-	ND	ND	ND	ND	<i>P. aeruginosa</i>
+	-	-	-	ND	A	A	A	<i>S. saprophyticus</i>

Key: +=Positive, -=Negative, A=Acid, G=Gas, ND= Not Done.

Table 6. Microscopy of urine deposits

Age	Appearance	WBC	RBC	Yeast	Epithelia cells	<i>T. vaginalis</i>
8-12	Amber, & cloudy	11-20	F	F	F, M	F
13-17	Amber, red & cloudy,	11-40	MD	F	M	F
18-22	Amber, red & cloudy	11-40, 40-	F, M	M	F	MD
23-27	Amber, red	11-40	F, MD, M	M	M	MD
28-32	Amber, red & cloudy	11-40, 40-	MD, M	M	MD	F
33-37	Red & cloudy	11-40, 40-	F, M, MD	M	F	M
38-42	Red & cloudy	11-40, 40-	MD	M	M	MD, M
43-47	Amber red & cloudy	11-40, 40-	M	M	M	MD
48-52	Amber, red & cloudy	11-40	M	M	F	MD

Key: F = Few, MD = Moderate, M = Many

In urine microscopy, white blood cells (pus cells) were found. Up to 10WBCs / HPF (high power field, i.e. using 40x objectives) was considered as few in number. Moderate number: 11-40/ HPF, and more than 40 WBC/ HPF, was considered as many.

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