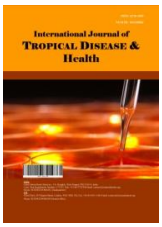

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Prevalence of Multi Drug Resistant Bacteria among Urinary Tract Infection Outpatients Visiting Madonna University Teaching Hospital, Elele, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author YNNN designed the study, performed laboratory analysis and along with author GAE performed statistical analysis and wrote the first draft of the manuscript. Author MSF interpreted the findings and wrote the first draft of the manuscript. Author DAF conceived the idea and supervised the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Urinary tract infections (UTIs) are among the most common infections in outpatients in Nigeria. However, antimicrobial drug resistance is a challenge in the treatment of UTIs and therefore, there is need for drug resistance surveillance to guide treatment.
Aim: This study assessed the prevalence and antimicrobial susceptibility patterns of UTIs in a Nigeria population.
Study Design: This was a cross-sectional study involving hospital outpatients.
Place and Date of Study: The study was conducted between September 2012 and February 2013 at Madonna University Teaching Hospital, Elele, Nigeria.

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Methodology: Urine sample collected from 115 randomly selected patients was screened by microbial culture for the presence of microbial pathogens. Antimicrobial susceptibility testing was done for the identified isolates to assess the effectiveness of some selected routinely used antibiotics.
Results: Among the 115 subjects, bacteria growth was observed in 49 subjects giving a prevalence of 42.6%. UTI was slightly higher in females (44.8%) than in males (40.4%). A total of 9 bacteria isolates of the following genus; *staphylococcus, klebsiella, pseudomonas, Escherichia, enterococcus and proteus species* were identified among which *Staphyloccus aureus* was the most prevalent. Ampicillin was the most effective antibiotics, followed by ciprofloxacin and erythromycin. *Staphylococcus aureus,* other *Staphylococcus species* and *Escherichia coli* were the most susceptible isolates. Most microbial pathogens were resistant to Nitrofurantoin, amoxicillin-clavulanic acid and amoxicillin.
Conclusion: Urinary tract infection showed a high prevalence of 42.6% which was predominant in females. *Staphylococcus aureus* was the most prevalent bacterial isolate. Ampicillin was the most effective antibiotics followed by ciprofloxacin while most pathogens were resistant to nitrofurantoin, amoxicillin-clavulanic acid and amoxicillin.

Keywords: Urinary tract infections; antibiotics; drug resistance; microbial pathogens.

1. INTRODUCTION

Urinary tract infections (UTIs) are the most common infections in outpatients with a prevalence of 50−60% which mostly affects adult women of reproductive age but is also common in males [1]. UTIs are the second most common type of infection in the human body as it accounts for over 8.1 million health care visits each year [2]. UTIs accounts for about 25 % of all infections and can occur in any population irrespective of the age group. Globally, about 150 million UTIs are recorded per year translating to a global cost of 6 billion US dollars [3]. In Nigeria, the prevalence of UTI ranges between 40-60% in the general population affecting mostly females [4,5].

UTIs are infections in the urinary tract which are commonly caused by microbes including viruses, fungi and bacteria but the latter is the most common cause of UTIs. The urinary tract constitutes the kidney, ureter, bladder and urethra. Infection in the urethra is called urethritis, in the bladder is known as cystitis while in the kidney is called pyelonephritis [6]. Following an infection in the urinary tract, microbial pathogens are commonly present in urine and women are more prone to UTIs than men [7]. UTIs could vary depending on the nature and type of infection. They could be classified as asymptomatic, uncomplicated, acute uncomplicated and recurrent uncomplicated UTIs. Symptoms of a UTI vary by gender, age and whether a catheter is present [8]. The common symptoms in females include an intense urge and frequency to urinate which is usually painful, burning feeling in the bladder or urethra during urination while older men and women are more likely to be weak, tired, shaky, and have muscle aches and abdominal pain. Generally, urine may look dark, cloudy, or bloody or have a foul smell. Fever is an uncommon symptom of UTIs especially in cystitis [9]. However, there might be fever in persons with a catheter, but also when the kidney is affected or pathogens have penetrated the prostate. In the case of kidney infection, symptoms may include nausea, vomiting as well as back and side pains [8].

About 95% of UTIs are caused by single bacterial species. The most common bacteria that cause UTIs include *Staphylococci, Klebsiella, Enterobacter, Pseudomonas, Candida, Proteus*, *Staphylococcus* and *Enterococci species* [10]. Among these pathogens, *Escherichia coli* is the most common cause of UTIs with a greater preponderance in an outpatient population [11]. Anaerobic organisms are not common cause of UTIs as are rarely pathogens in the urinary tract [12]. Moreover, *Staphylococcus saprophyticus* tends to cause infection in young women of a sexually active age which may be sexually transmitted [13]. Also, *Staphylococci species* are a common cause of urinary tract infection in some reports [14].

Since UTIs are caused by mostly bacteria, bacterial-fighting drugs commonly known as antibiotics, antibacterial or antimicrobial are used to treat UTIs. There exist several antibiotics for the treatment of UTIs which act over a broad spectrum of microbes [15,16]. These

antibiotics include nitrofurantoin, trimethoprim-sulfamethoxazole, pivmecillinam, fosfomycin, amoxicillin and ampicillin, amoxicillin-clavulanic acid, cephalosporins such as cefdinir cefaclor, proxetil and cefpodoxime. Also, fluoroquinolones including ofloxacin, ciprofloxacin, and levofloxacin are used for cystitis. For pyelonephritis, ceftriaxone (400 mg) or aminoglycoside such as gentamicin and tobramycin can be used in addition to oral ciprofloxacin [17]. One of the biggest challenges in the treatment of UTIs is the development of resistance by the pathogens against antibiotics. Generally, fluoroquinolones are the preferred initial agents for the treatment of UTI in areas where resistance is common [18] as these antibiotics have shown high clinical and bacteriological cure rates with low rates of resistance by most uropathogens [18,19]. Due to the rise of drug resistance by pathogens against antibiotics in the treatment of UTIs, it has been recommend by WHO that physicians should obtain laboratory information on local resistance spectrum of organisms before UTI treatment and that periodic surveillance should be conducted to monitor changes in susceptibility of uropathogens [14]. Therefore, the aim of this study was to assess the prevalence and antimicrobial susceptibility patterns of UTIs in a Nigeria population.

2. MATERIALS AND METHODS

2.1 Study Design and Population

This was a cross-sectional study that involved outpatients visiting Madonna University Teaching hospital (MUTH), Elele, Rivers State of Nigeria between September 2012 and February 2013. Ethical clearance was obtained from MUTH Institutional Research Ethics Committee and also patients provided written informed consent before recruitment into the study. Outpatients visiting the hospital without fever or complications were recruited for the study. Patients who were ill, having complications and admitted in the hospital were excluded from the study.

2.2 Sample Collection and Microbiological Analysis

Isolation and identification of isolates from urine samples were aseptically carried out using standard microbiology techniques as described by Cheesbrough [20]. A sterile micropipette was used to transfer 0.1 ml of urine sample on different selective media including MacConkey agar plates and CLED (cysteine-, lactose- and electrolyte-deficient). Eosin methylene Blue (EMB) agar plates were used to isolate *E. coli* and *Enterobacter spp*. As lactose non fermented bacteria, the growth of *Proteus spp* was cultured on MacConkey agar. Subsequent culture on blood agar and Cetrimide agar after isolation on MacConkey agar were used for the isolation of *Staphylococcus spp* and *Pseudomonas spp* respectively. The raw samples were spread onto Nutrient Agar to determine total bacterial count. The agar plates were incubated at 37^{o}C for 18-24 hours and were counted and the mean expressed as CFU/ml. Bacteria were identified based on their colony morphology, grams staining, motility test and biochemical tests such as catalase test, oxidase test, indole test, citrate test, Methylred-Voges Proskauer (MRVP) test, hydrogen sulphide production test, urease test, and sugar fermentation.

2.3 Antibiotic Susceptibility Test

Bacteria isolates were subjected to *in-vitro* susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [21]. In brief, a single colony of each isolate was inoculated into 2 ml of Mueller-Hinton broth, and incubated at 37°C for 4 hours. The culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and spread evenly over the entire surface of Muller-Hinton agar. After the inoculums were dried, antibiotic impregnated discs of appropriate concentrations (ciprofloxacin (5µg), amoxicillin (10µg), erythromycin (10µg), ampicillin (10µg), amoxicillin-clavulanic acid (10µg) and nitrofurantoin (300µg)) were placed aseptically over the surface of the agar at appropriate spatial distance of 5 millimetre (mm). Plates were then inverted and incubated at 37°C. After 24 hours, plates were examined and the diameters in mm of the zones of inhibition were measured and interpreted as susceptible, intermediate and resistant on the basis of the critical points recommended by the CLSI.

2.4 Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 16 was used to analysis data. Data was presented in figure, tables and expressed in

percentages. Chi-square test was used to compare difference between qualitative variables. A *p*-value ≤0.05 was considered statistically significant.

3. RESULTS

A total of 115 patients were enrolled for this study. The number of patients with bacteria growth was 49 giving a prevalence of 42.6%. From 49 participants with bacteria growth, there were 64 bacteria isolates with some of the samples showing poly microbial growth of more than one bacterial isolates. Nine bacteria species were isolated from the 64 isolates. The prevalence of the various bacterial infections ranged from 5.2-20%. *Staphylococcus aureus* was significantly (p = 0.032) the most frequent isolate (23) having a prevalence of 20%, followed by *Staphylococcus species* with 8 isolates and the least was *Pseudomonas aeroginosa* with just one isolate (Table 1).

Table 1. Occurrence and prevalence of bacterial isolates from urine cultures of patients

Sample size (115)	Bacterial infections	Prevalence (%)
Bacteria urinary tract infection	49	42.6
Staphylococcus aureus	23	20.0
Staphylococcus species	8	7.0
Klebsiella species	4	3.5
Pseudomonas species	4	3.5
Pseudomonas aeroginosa	1	0.9
Escherichia coli	7	6.1
Enterococcus species	8	7.0
Enterococcus faecalis	3	2.6
Proteus species	6	5.2
Total	64	55.8

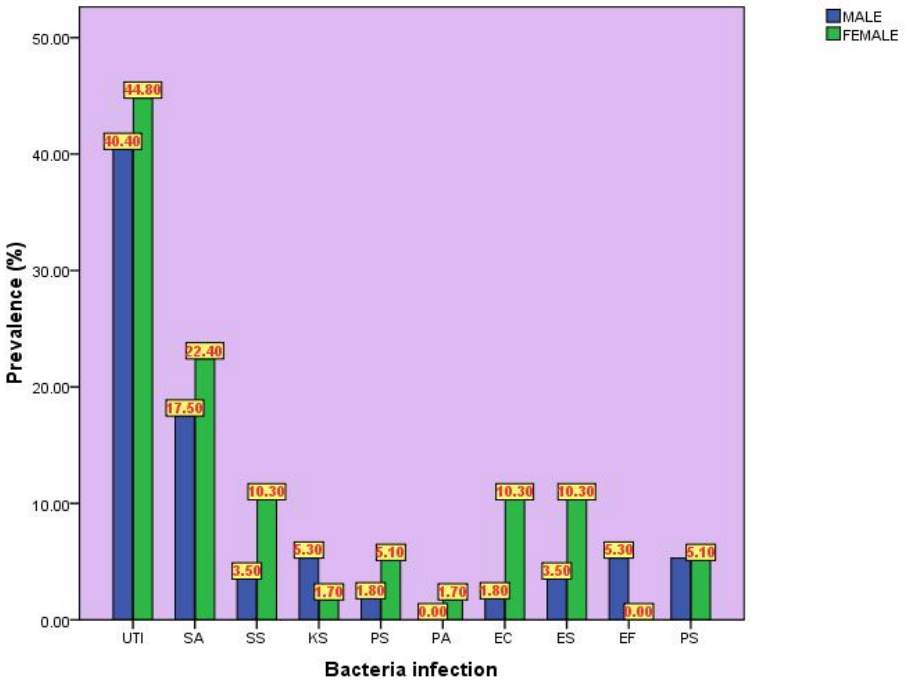

Fig. 1. Percentage distribution of urinary tract infections in male and female patients

EC: Escherichia coli, EF: Enterococcus faecalis, ES: Enterococcus species, KS: Klebsiella species, PA: Pseudomonas aerogenosa, PS: Pseudomonas species, SA: Staphylococcus aureus, SS: Staphylococcus species, UTI: Urinary tract infection

Among the 115 study participants, 57 were males and 58 females. The prevalence of bacterial urinary tract infection was slightly higher in females (44.8%) compared to males (40.4%) though not significantly (p = 0.5) different. In both males and females, *Staphylococcus aureus* was the bacteria species most frequently isolated with 10 and 13 isolates respectively. However, *Staphylococcus species, Escherichia coli* and *Enterococcus species* were significantly higher ($p \leq 0.05$) in females compared to males (Fig. 1).

The antimicrobial susceptibility pattern of all the six antibiotics (amoxicillin, erythromycin, ampicillin, ciprofloxacin, amoxicillin-clavulanic acid and nitrofurantoin) against the various isolates showed sensitive, intermediate and resistant patterns. In general, almost all the bacteria species showed some resistance to at least one of the antibiotics. The antimicrobial susceptibility pattern varied across the various bacteria isolates. *Staphylococcus aureus,* other *Staphylococcus species* and *Escherichia coli* were the most susceptible to almost all the antibiotics. *Klebsiella species*, *Pseudomonas species*, *Enterococcus species*, *Enterococcus faecalis* and *Proteus species* were less susceptible, showing high resistance of up to 100% to the antibiotics. Among the antibiotics, ampicillin was the most effective antibiotics which most of the isolates were susceptible to, followed by ciprofloxacin and erythromycin. Nitrofurantoin and amoxicillin were the least effective against most of the microbial isolates. All the other antibiotics (amoxicillin, erythromycin, amoxicillin-clavulanic acid and nitrofurantoin) showed similar susceptibility pattern and were effective to at least one isolate. Most microbial pathogens were resistant against nitrofurantoin, amoxicillin-clavulanic acid and amoxicillin (Table 2).

4. DISCUSSION

UTI is a public health problem of concern in Nigeria as the infection is very common in this country generally affecting individual of both sex and in all age groups with increased morbidity and associated health complications. In this study, the prevalence of UTI was 42.6%. This shows that UTI is highly prevalent in this population. Previous studies in Nigeria have also shown high prevalence of UTI. A study in Ebonyi State showed a 55% prevalence of UTI in pregnant women [22]. A prevalence of 78% was observed in a study on female students in South Eastern Nigeria [23]. Another study in Nigeria showed approximately 40% prevalence in a rural population [4]. A study in India showed a 53.82% prevalence of UTI [24]. However, the prevalence of UTI in this study was higher than that of other studies in Nigeria: Keffi (20%) [25], Ogun State (25%) [26], Southeast (13.8%) [27] and Imo State University (28%) [28] that recorded a low prevalence. More so, the prevalence of UTI in this study was higher in females (44.8%) than in males (40.4%). This finding supports the already known fact that UTIs generally affect females than males [29]. This may be so as females have wider and shorter urethra which is proximate to the anus. Also, females lack prostatic fluid which acts as an antimicrobial agent in males. More so, females have moist and warm urethra which may support the growth of bacteria than in males [30]. In addition, pathogens may easily be introduced into the urinary tract from the female reproductive system during sexual intercourse [31].

Several pathogens have been responsible for UTI, both gram negative and positive bacteria. In this study, 9 bacteria isolates of the following genera; *staphylococcus, klebsiella, pseudomonas, Escherichia, enterococcus and proteus species* were responsible for the infection. Among these pathogen, *Staphyloccus aureus* was the most prevalent (20%). This finding is in line with other studies which have shown *S. aureus* to be the most prevalent in UTI [32,33]. However, this study disagrees with other reports which have shown *E. coli* to be the most prevalent pathogen in UTI in a study in India [24], Ethopia [34], and Nigeria [4,22]. In this study, *E. coli* had a low prevalence of 6.1%. The reason for the high prevalence of *S. aureus* could be as a result of poor hygienic and sanitary conditions of toilets and bathrooms making females to easily contract them when urinating or defecating. *Pseudomonas aeroginosa* had the lowest prevalence in the population as it was present in only one participant.

Antimicrobial drug resistance is the main challenge faced in treating UTIs [35]. Several pathogens have developed resistance against antibiotics and therefore it has become necessary for epidemiological surveillance to screen antibiotics against UTI pathogens to identify reliable and efficacious antibiotics for proper treatment. In this study, ampicillin was the most effective antibiotics, followed by ciprofloxacin and erythromycin. *Staphylococcus aureus,* other *Staphylococcus species* and *Escherichia coli* were the most susceptible to almost all the antibiotics. Previous studies have shown ciprofloxacin to be highly sensitive

Table 2. Susceptibility pattern of antiobiotics against various bacteria isolates

Bacteria isolates	N		AMO	ERY	AMP	CIP	AMC	NIT
Staphylococcus aureus	23	S	1 (4.3)	6 (26.1)	7 (30.4)	9 (39.1)	3 (13.0)	1 (4.3)
		I	9 (39.1)	12 (52.2)	16 (69.5)	10 (43.5)	7 (30.4)	8 (34.8)
		R	13 (56.5)	5 (21.7)	0 (0)	4 (17.4)	13 (56.5)	14 (60.9)
Staphylococcus species	8	S	1 (12.5)	0 (0)	4 (50)	3 (37.5)	0 (0)	0 (0)
		I	2 (25)	5 (62.5)	2 (25)	2 (25)	2 (25)	2 (25)
		R	5 (62.5)	3 (37.5)	2 (25)	3 (37.5)	6 (75)	6 (75)
Klebsiella species	4	S	1 (25)	0 (0)	3 (75)	0 (0)	0 (0.0)	0 (0.0)
		I	0 (0)	3 (75)	1 (25)	3 (75)	0 (0)	2 (50)
		R	3 (75)	1 (25)	0 (0)	1 (25)	4 (100)	2 (50)
Pseudomonas species	4	S	0 (0.0)	0 (0.0)	2 (50)	0 (0.0)	0 (0.0)	0 (0.0)
		I	1 (25)	2 (50)	2 (50)	2 (50)	1 (25)	1 (25)
		R	3 (75)	2 (50)	0 (0)	2 (50)	3 (75)	3 (75)
Pseudomonas aerogenosa	1	S	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
		I	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)
		R	0 (0.0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
Escherichia coli	7	S	0 (0.0)	1 (14.3)	4 (57.1)	2 (28.6)	1 (14.3)	1 (14.3)
		I	3 (42.9)	3 (42.9)	3 (42.9)	3 (42.9)	0 (0.0)	2 (28.6)
		R	4 (57.1)	3 (42.9)	0 (0)	2 (28.6)	6 (85.7)	4 (57.1)
Enterococcus species	8	S	0 (0)	0 (0)	2 (25)	4 (50)	0 (0)	0 (0)
		I	3 (37.5)	4 (50)	6 (75)	1 (12.5)	0 (0)	1 (12.5)
		R	5 (62.5)	4 (50)	0 (0)	3 (37.5)	8 (100)	7 (87.5)
Enterococcus faecalis	3	S	0 (0)	0 (0)	2 (66.7)	0 (0)	0 (0)	0 (0)
		I	1 (33.3)	1 (33.3)	1 (33.3)	2 (66.7)	1 (33.3)	1 (33.3)
		R	2 (66.7)	2 (66.7)	0 (0)	1 (33.3)	2 (66.7)	2 (66.7)
Proteus species	6	S	0 (0)	0 (0)	3 (50)	2 (33.3)	0 (0)	1 (16.7)
		I	1 (16.7)	2 (33.3)	3 (50)	0 (0)	2 (33.3)	1 (16.7)
		R	5 (83.3)	4 (66.7)	0 (0)	4 (66.7)	4 (66.7)	4 (66.7)

Note: N: Number of isolates; S: Sensitive; I: Intermediate; R: Resistance; AMO: Amoxicillin; ERY: Erythromycin; AMP: Ampicillin; CIP: Ciprofloxacin; AMC: Amoxicillin-clavulanic acid; NIT: Nitrofurantoin

against microbial pathogens responsible for UTIs in Ethiopia [34] and Nigeria [22]. Ciprofloxacin is a member of the fluoroquinolones and has been effective against a wide range of organisms [18]. Nitrofurantoin and amoxicillin were the least effective against most of the microbial isolates. Ampicillin was most sensitive against most of the isolates while ciprofloxacin was sensitive against *S. aureus* and *Enterococcus species.* Most microbial pathogens were resistant against nitrofurantoin, amoxicillin-clavulanic acid and amoxicillin. *S. aureus* has been shown to exhibit high rate of resistance to amoxicillin [36]. The high level of resistance by pathogens to these antibiotics could probably be because they have been marketed for a long time in Nigeria, thus allowing microorganisms a longer time to develop resistance mechanisms against the antibiotics. More so, the poor usage of the antibiotics by patients who can easily purchase these antibiotics by street vendors without any proper diagnosis and defined dosage has led to resistance and the ineffectiveness of these antibiotics. This can equally be attributed to easy access and uncontrolled sales and production of antibiotics by unlicensed pharmacies and personnel across Nigeria.

5. CONCLUSION

Urinary tract infection showed a high prevalence of 42.6% which was predorminant in females. *Staphylococcus aureus* was the most prevalent bacteria isolate while *Escherichia coli* showed a

low prevalence. Ampicillin was the most effective antibiotics followed by ciprofloxacin as were sensitive against most bacteria isolates while most pathogens showed resistance to nitrofurantoin, amoxicillin-clavulanic acid and amoxicillin with *Klebsiella species, Enterococcus species* and *Proteus species* showing the greatest rate of resistance. The increase rate of resistance to antibiotics is of major health concern and therefore, there is need for the enforcement of policies on the proper production, marketing and monitoring of the sales and usage of antibiotics in Nigeria.

CONSENT

Only subjects who gave written informed consent were recruited for the study.

ETHICAL APPROVAL

Ethical permission was sought from Madonna University Teaching Hospital (MUTH) Institutional Research Ethics Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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