



(RESEARCH ARTICLE)



## Sensitivity of microorganisms associated with urinary tract infections to some antibiotics among patients in hospitals within Makurdi metropolis, Nigeria

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### Abstract

Urinary Tract Infections (UTI) constitute serious health problems affecting all ages and sexes. The microorganisms implicated in urinary tract infections from four different hospitals in Makurdi Metropolis, Nigeria were investigated. Two hundred midstream urine samples were collected from both male and female patients for analysis by Kirby Bauer's method following the definition of the National Committee of Clinical Laboratory Standards. The microorganisms present in the urine samples that were identified to cause UTI in four hospitals within Makurdi Metropolis included *Escherichia coli* (43.8%), *Staphylococcus aureus* (23.9%), *Klebsiella* spp (10.74%), *Streptococcus pyogenes* (9.09%), *Pseudomonas aeruginosa* (8.26%) and *Proteus species* (4.13%). The result showed that 39.5% of the urine samples collected had no microorganism. The isolates in female patients were higher (77%) compared to the male patients (44%). Antimicrobial drugs such as gentamicin and erythromycin tested on the isolated microorganisms showed higher efficacy to all isolates, while streptomycin, tetracycline and ampicillin showed lower efficacy to the probable isolates. This research showed that *Escherichia coli* are the major cause of UTI.

**Keywords:** Urinary; Tract; Infections; Microorganisms; Patients; Antimicrobial

### 1. Introduction

Urinary Tract system is composed of the kidneys, ureters, bladder and urethra (and prostate gland in males). Its main function is excretion of the urine. The urinary tract consists of various organs of the body that produce, store and get rid of urine. The tract just like any other system can be infected and an infection of any part of the system can spread to the whole system [14]. Urinary tract infection (UTI) is defined as an infection in any part of the urinary system accompanied by symptoms. They are severe health problems which affect 150 million people each year worldwide [21;3].

The most common causes of urinary tract infections are by *Escherichia coli* strains that usually inhabit the colon. However many other bacteria such as *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Proteus*, *Staphylococcus*, *Mycoplasma*, *Chlamydia*, *Serratia* and *Neisseria* species can cause infections. In addition, fungi (*Candida* and *Cryptococcus species*) and some parasites (*Trichomonas* and *Schistoma*) also cause UTI. Urinary tract infections (UTI) may also occur as a result of lapses in hygiene standard both in individual and communal levels [22]. Incomplete emptying of the bladder during urinating can cause urinary tract infection [2]. UTIs are also sexually transmitted as a result of injury or irritation to the urethral opening during vigorous sexual intercourse.

Studies have shown that urinary tract infections pose a serious health problem affecting millions of people every year. Women are more susceptible to urinary tract infections than men [18]. According to [6], about 50% of adult women experience urinary tract infections at some point in their life. UTI causes severe health problems ranging from dysuria (pain and burning sensations when urinating) while some infections go unnoticed. For someone who is experiencing a

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urinary tract infection for the first time, the symptoms can be very frightening. On the other some persons with UTI may not experience any symptoms at all. They cause organ damage and even death. Urinary tract infections account for a significant part of the work load in Clinical Microbiology Laboratories and in particular *Escherichia coli* remain the most frequent cause.

### **1.1. Objectives of the Study**

The aim of the study was to isolate and identify the microorganisms associated with urinary tract infection (UTI) and to determine their antimicrobial susceptibility patterns as well as the rate of UTI infection among patients in Makurdi metropolis.

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## **2. Material and methods**

### **2.1. Study Area**

The study was carried out in Makurdi, Nigeria. Samples were collected from patients who attended the four major hospitals within the town as at the period of the study. These are; Federal Medical Centre, Bishop Murray Medical Centre, High level, Immaculate Hospital High level and General Hospital Northbank all within Makurdi Metropolis respectively.

### **2.2. Materials**

Equipment used includes: Incubator, Refrigerator, Bunsen burner, Weighing balance, Wireloop, autoclave, glass wares such as conical flask, sterile universal bottles, petridishes, test tubes and test tube tracks were also used in the study as well as sterile dispensing pipettes, plastic tubes, sterile Pasteur pipettes.

### **2.3. Media**

CLED (Cysteine Lactose Electrolyte Deficient) agar, Nutrient agar, blood agar, Nutrient broth and 0.5 McFarland standards.

### **2.4. Antibiotics**

The antibiotics used for susceptibility tests were; Ampicillin, Gentamicin, Erythromycin, Streptomycin and Tetracycline.

### **2.5. Sterilization of Materials**

Test tubes, petri dishes, wireloop and conical flask were all sterilized using the autoclave at 121 °C for 15 minutes. This is important to kill all micro-organisms including spore formers that may adhere to the surfaces of these materials.

### **2.6. Sampling Technique**

Two hundred (200) samples of mid-stream urine were collected randomly within the period of one month from patients attending the four hospitals. Sterile bottles were given to each patient for sample collection. The patients were instructed on how to aseptically collect mid-stream urine which was used for the analysis. The samples were properly labelled and parameters such as questionnaires were also issued to the patients.

### **2.7. Isolation of Pathogens**

Samples collected from each patient was analysed by inoculating each on CLED medium and blood agar. The plates were then incubated at 37 °C for 18–24 hours for growth to occur.

### **2.8. Identification of Isolates**

After 24 hours of incubation, the plates were observed for visible growth. Discrete colonies were picked for further identification. The culture appearance and morphology of the isolate were also appropriately noted to aid identification of the isolates. Pure isolates were obtained from nutrient agar plates following overnight incubation and biochemical test carried out to properly identify the bacteria.

## 2.9. Antibiotic Susceptibility Test

A colony of the identified bacteria was picked and inoculated into nutrient broth in a test tube and incubated for 24 hours for growth of the bacteria. After over the night growth, the suspension density was standardized using 0.5 McFarland standards. A sterile cotton swab was dipped into the suspension and t streak the entire surface of a Nutrient agar plate. The entire agar surface was evenly streaked, after which antibiotic impregnated discs of known concentration were placed on the agar surface and incubated at 37 °C for 24 hours. The antibiotics used for the test were Gentamicin (10 µg), Streptomycin (10 µg), Tetracycline (30 µg), Erythromycin (15 µg) and Ampicillin (10 µg).

## 2.10. Determination of Susceptibility Pattern

After 24 hours of incubation, the diameter of the zones of inhibition found on the plates was measured in millimetres. These measurements were then compared with interpretation criteria published by the Clinical Laboratory Standard Institute (CLSI) to determine whether the bacteria were sensitive or resistant to the antibiotics used.

## 2.11. Statistical Analysis

One way ANOVA was used to analyse the age distribution of patients in the four hospitals together with the percentage of singles and married couples.

## 3. Results

Table 1 showed the morphological characteristics of these isolates as evidenced by their shapes, size in diameter, colours and other confirmatory test.

**Table 1** The morphological characteristics of isolated organisms from the four hospitals in Makurdi

Isolates	Morphological characteristics of the colonies
<i>Escherichia coli</i>	Large colonies, elevated, yellow, opaque, with slightly dark centre.
<i>Staphylococcus aureus</i>	Deep yellow colonies, spherical in shape (2–3 mm) in diameter and arranged in irregular clusters.
<i>Klebsiella pneumonia</i>	Large colonies, yellow or yellowish–white, highly mucoid and elevated
<i>Streptococcus pyogenes</i>	Small colonies (0.4mm), yellow and opaque
Proteus spp	Blue colonies, translucent with irregular edges, slightly elevated
Pseudomonas spp	Smooth round colonies with a fluorescent greenish colour, produces sweet or grape like colour.

Table 2 showed the occurrence of bacteria isolates collected from urine samples in both male and female patients in the four hospitals. They included; *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. *Escherichia coli* had the highest occurrence (43.8%) and *Proteus mirabilis* had the lowest occurrence (4.13%).

**Table 2** Frequency of bacterial isolates from urine samples in the four hospitals

Isolates	Occurrence
<i>Escherichia coli</i>	53(43.8%)
<i>Staphylococcus aureus</i>	29(23.9%)
<i>Klebsiella pneumonia</i>	13(10.74%)
<i>Streptococcus pyogenes</i>	11(9.09%)
<i>Pseudomonas aeruginosa</i>	10(8.26%)
<i>Proteus mirabilis</i>	5(4.13%)
<b>Total</b>	<b>121(100%)</b>

Table 3 showed the percentage occurrence of male and female patients within the ages of 15–19, 20–24, 25 and above in the four hospitals having positive isolates, with *Escherichia coli* having the highest occurrence in female. Statistical

analysis using ANOVA showed that there was no significant difference between the various age groups studied as  $F_{cal}=6.02 < F_{Tab}=9.55$

**Table 3** Age distribution of probable isolates among male and female patients from the four hospitals in Makurdi, Nigeria

Age group	Number with positive isolates	
	Male (%)	Female (%)
15–19	5(11.36)	14(18.18)
20–24	9(20.45)	22(28.57)
25 and above	30(68.18)	41(53.25)
<b>Total</b>	<b>44(100)</b>	<b>77(53.25)</b>

Table 4 showed the distribution of probable isolates among single males and females in the four hospitals within Makurdi Metropolis. *Escherichia coli* had the highest occurrence in the males (40.0%) and in females (50.0%), while *Proteus mirabilis* had the lowest occurrence in males (8.0%) and 5.36% in females.

**Table 4** The Distribution of probable isolates among single male and female patients from four hospitals in Makurdi, Nigeria.

Isolates	Occurrence	
	Male (%)	Female (%)
<i>Escherichia coli</i>	10(40)	28(50)
<i>Staphylococcus aureus</i>	2(8)	8(14.29)
<i>Klebsiella spp</i>	4(16)	7(12.5)
<i>Streptococcus pyogenes</i>	4(16)	5(8.93)
<i>Pseudomonas spp</i>	3(12)	5(8.93)
Proteus	2(8)	3(5.36)
<b>Total</b>	<b>25 (100)</b>	<b>56 (100)</b>

$$F_{cal} = 2.39 < F_{Tab} = 4.39$$

Table 5 showed the distribution of of probable isolates among married males and females in the four hospitals in Makurdi town. *Escherichia coli* had the highest occurrence in both males (31.25%) and females (41.67%) patients. Statistical analysis using ANOVA showed that there was significant difference among male and female patients in terms of urinary tract infections ( $F_{cal}=13.25 > F_{Tab}=4.39$ ).

**Table 5** The Distribution of probable isolates among married males and females from the four hospitals.

Isolates	Occurrence	
	Male (%)	Female (%)
<i>Escherichia coli</i>	5(31.25)	10(41.67)
<i>Staphylococcus aureus</i>	9(56.25)	10(41.67)
<i>Klebsiella spp</i>	1(6.25)	1(4.17)
<i>Streptococcus pyogenes</i>	1(6.25)	1(4.17)
<i>Pseudomonas spp</i>	–	2(8.33)
Proteus	–	–
<b>Total</b>	<b>16(100)</b>	<b>24(100)</b>

$$F_{cal} = 13.25 > F_{Tab} = 4.39$$

Table 6 showed the antibiogram of gram positive isolates in the four hospitals with gentamicin, tetracycline and ampicillin having the highest efficacy.

**Table 6** Antibiogram of gram positive isolates from four hospitals in Makurdi town, Nigeria

Isolates	Gentamicin		Streptomycin		Erythromycin		Tetracycline		Ampicillin	
	(R%)	(S%)	(R%)	(S %)	(R%)	(S%)	(R%)	(S%)	(R%)	(S%)
S. aureus	6(20.69)	23(79.31)	6(20.69)	23(79.31)	22(75.86)	7(24.14)	6(20.69)	23(79.31)	7(24.14)	22(75.86)
S.pyogenes	2(18.18)	9(81.82)	9(81.82)	2(18.18)	3(27.27)	8(72.73)	2(18.18)	9(81.82)	10(90.91)	1(9.09)

Table 7 showed the antibiogram of gram negative isolates in the four hospitals. Gentamicin, erythromycin and ampicillin had the highest efficacy. Streptomycin and tetracycline had the lowest efficacy.

**Table 7** Antibiogram of gram negative isolates in from four hospitals in Makurdi town.

Isolates	Gentamicin		Streptomycin		Erythromycin		Tetracycline		Ampicillin	
	(R%)	(S%)	(R%)	(S%)	(R%)	(S%)	(R%)	(S%)	(R%)	(S%)
E.coli	15(28.3)	38(71.69)	42(79.25)	11(20.75)	22(41.51)	31(58.49)	44(83.2)	9(16.98)	30(56.60)	23(43.40)
P.aeruginosa	3(30)	7(70)	1(10)	9(90)	3(30)	7(70)	4(40)	6(60)	–	10(100)
Klebsiella spp	3(23.7)	10(76.92)	12(92.31)	1(7.69)	4(30.77)	9(69.23)	12(92.31)	1(7.69)	4(30.77)	9(69.23)
Proteus spp	–	5(100)	3(60)	2(40)	–	5(100)	2(40)	3(60)	1(20)	4(80)

#### 4. Discussion

The microorganisms causing urinary tract infections in both male and female patients attending four different hospitals in Makurdi town revealed that 60.5% showed positive isolates while 39.5% of the urine samples had no isolates. This indicated the high occurrence of urinary tract infections among patients attending the four selected hospitals in Makurdi. Among the positive isolates in the urine samples collected, *Escherichia coli* (43.8%) had the highest percentage of occurrence. This agrees with [1] who reported that *Escherichia coli* were the predominant isolates in the urine samples examined. This underscores that infections due to *Escherichia coli* were very high among patients attending the four hospitals in Makurdi. The high prevalence of the bacteria in the urine samples could have been as a result of faecal flora gaining access into the bladder [6]. [9] suggested that *E.coli* is the dominant pathogen in both males and females and it accounts for approximately 85% of community acquired cases and 50% of nosocomial UTIs. Also, [16] suggested that the most common bacteria found in the UTIs is *Escherichia Coli*.

From the result of the research that was carried out in Benue State on UTI among patients of NKST hospital Mkar, the microscopic elements were counted in urine samples collected from male and female patients. While the urine samples collected from male patients had less microscopic elements, this could be as a result of higher microbial load in the females than the males, since the presence of many microbial elements is an indication of an infection in most cases from the result. The genera of bacteria isolated from this study include; *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. The percentage of gram positive organisms was higher (77%) than the gram negative organisms (22.5%), *Staphylococcus aureus* had the highest prevalence (65%) and this could be as a result of haematogenous or ascending infections. The next organism was *Staphylococcus aureus* (23.9%), followed by *Klebsiella pneumoniae* (10.74%), *Streptococcus pyogenes* (9.09%), *Pseudomonas aeruginosa* (8.26%) and *Proteus mirabilis* had the least (4.13%). This result agrees with [10] who reported that *Escherichia coli* is the most common organism, isolated from urine samples, followed by *Staphylococcus aureus* and *Klebsiella spp.*

The age distribution of male and female patients from the four hospitals and prevalence of infection among them were ages 15–19 years for male (11.36%) and female (18.18%), ages 20–24 years for males (20.45%) and females (28.57%) and ages 25 years and above for males (68.18%) and females (53.25%) respectively. [8] reported that the incidence of UTI among teenagers is due to urethra trauma from sex. However, among women between the age of 15–24 years, there is a peak incidence of UTI suggests that hormonal and anatomical changes may be associated with UTI among them [17]. Though this particular research disagrees with it. This result is similar to the findings of [13] who reported that women are more prone to urinary tract infections than men. Also there are several factors and abnormalities of UTI that interfere with natural resistance to infections, these factors include sex, age, disease, hospitalization and obstruction [7]. Cases of mixed infections could have originated from gastrointestinal or genitourinary fistulae, bladder outlet obstruction, neurogenic bladder and indwelling catheters. Finally, reduced fluid intake helps to keep the bladder active and bacteria free could have enhanced their susceptibility of these infective organisms. According to [19] who stated that in adult women, UTI occurs in about 20% between 20–50 years at one stage in their life time. Statistically, there is no significant difference between the age distribution of single male and female patients with positive isolates ( $P$  values  $< 0.05$ ). However, there was significant difference between the isolated organisms in married male and female patients ( $P < 0.05$ ). [19] reported that among women ages 20–50, the unmarried ones tend to have lower rates of bacteriuria while the rate increases with parity in the married ones.

Antibiogram of gram positive isolates in the four hospitals showed that *Staphylococcus aureus* was sensitive to gentamicin (79.31%), Streptomycin (79.31%), tetracycline (75.86%) and ampicillin (75.86%) respectively. However, *Staphylococcus aureus* are highly resistant to erythromycin (75.86%). *Streptococcus pyogenes* was sensitive to gentamicin (81.81%), tetracycline (81.81%) and erythromycin (72.73%), respectively. But this organism was highly resistant to streptomycin (81.81%) and ampicillin (90.91%). This resistance may be due to the widespread use and more often misuse of antimicrobial drugs which has led to the emergence of highly resistant bacteria [15]. The antibiotic susceptibility of gram-negative isolates from the four hospitals showed that all isolates were sensitive to gentamicin in the following percentages; *Proteus mirabilis* (100%), *Klebsiella pneumonia* (76.92%), *Escherichia coli* (71.69%), *Pseudomonas aeruginosa* (70%), respectively. *Escherichia coli* showed resistance to streptomycin, tetracycline and ampicillin. Other gram-negative isolates from this study were sensitive to erythromycin such as *Klebsiella pneumonia* (69.23%) and *Proteus mirabilis* (80%). *Pseudomonas aeruginosa* showed sensitivity to the five antibiotics with ampicillin possessing the highest efficacy (100%). [5] also revealed the resistance of commonly used antibiotics to clinical isolates.

The above antimicrobial drugs are the commonly prescribed antibiotics in hospitals even before the result of urine analyses; and also they are readily available in the market without prescription and are very cheap. Inappropriate

practices like misuse and abuse of antibiotics and unskilled practitioners can also lead to emergence of resistance in bacteria. Expired antibiotics, self-medication, counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates [20]. In developing countries like Nigeria, self-medication is a common practice and this might probably be a major cause of antibiotic resistance in clinical isolates since patients only think of going to the hospitals when they are unable to treat themselves.

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## 5. Conclusion

The susceptibility and resistance profile of all isolates in the study have shown that gentamicin and erythromycin possess the highest efficacy, while streptomycin, ampicillin and tetracycline possess lower efficacy which are the frequently used drugs in most of the developing world today. More research therefore should be carried out on UTI so as to be able to come up with therapies that will help stop and control these organisms to the barest minimal level, else bacterial resistance would continue to be the greatest threat to the treatment of UTIs.

### *Recommendations*

- Proper cleaning up after urination and bowel movement should be encouraged in order to prevent bacteria from the anus gaining access to the genital regions.
- Both males and females should be educated on the practice of personal hygiene, mostly around the genitals and ensure that panties and underwear are germs free.
- People should be enlightened on the dangers of unsafe sex and the need to practice total abstinence. This will help to reduce the risk of acquiring UTI through sex.
- Damaged toilets and bathrooms should be repaired.
- People with noticeable symptoms of urinary tract infections should see the physician for proper check-up before administering the rightful antibiotic.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

Authors have declared that there is no conflict of interest.

### *Statement of ethical approval*

Ethical approval for this study was received from the Medical Directors in charge of each of the hospitals from which the samples were collected. The hospitals included Federal Medical Centre, Bishop Murray Medical Centre, General hospital North bank and Immaculate hospital all Makurdi, Nigeria. The Management committee of the four hospitals did not see any adverse ethical problem arising from their methodology. The authors were therefore permitted to go on with the study. The Authors were advised to stick strictly to their design and work within the frame work of the time given to them.

### *Statement of informed consent*

A letter of introduction was given to the authors by the Department of Microbiology, Federal University of Agriculture, Makurdi introducing the authors to carry out a research on Sensitivity of microorganisms associated with urinary tract infections to some antibiotics among patients in hospitals within Makurdi metropolis, Nigeria. The Health Research Ethics Committee of the four hospitals after thorough scrutiny, resolved that the authors be given access to the hospitals' Laboratory and be assisted in the study for successful conduct of their research.

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