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# Assessment of Bacteriological and Antibiogram of Uropathogens among Students in the Faculty of Health Sciences, Imo State University, Owerri

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The Assessment of bacteriological and antibiogram of uropathogens among students in the faculty of Health Sciences, Imo State University, Owerri with the aim to determine the prevalence of the isolates and to test which antibiotic has the greatest antagonistic activity against the different isolates in the study area. Fifty (50) students made up of both sexes were examined using their midstream urine samples as specimen. The study took note of the students' age, sex and department. The urine samples were cultured on Nutrient agar, Mannitol salt agar, and MacConkey agar using pour plate method and were incubated at $37^{o}$C for 24 hours. After which the total bacterial counts were carried out and based on the count, it was categorized as being significant, suspected and non-significant. The colonies were then sub cultured for further identification. The pure isolates produced were stained using Gram stain, examined microscopically and further tested using relevant biochemical tests. It was found that a total of 35 bacteria were isolated which includes *E.coli* 14(40%) being the most predominant organism, followed by *Staphylococus aureus* 13(37.1%), *Proteus mirabilis* 7(20%) and *Pseudomonas aeruginosa* 1(2.9). Out of the fifty (50) urine samples investigated, 24 samples were significant for UTI ($10^5$ CFU/ml), 2 samples were suspected for UTI ($10^2$ – $10^4$ CFU/ml), while 24 were not significant for UTI (below $10^2$CFU/ml). Based on departmental studies, the subjects from the Department of Nutrition and Diatetics and

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Optometry had the highest significance of UTI, (60%) respectively. The results of susceptibility tests showed that Imipenem was the most effective antibiotic in inhibiting the bacterial growth (98.8% of antibiotic activity). The present study therefore, revealed that the urine samples collected from students in Faculty of Health sciences, Imo State University, Owerri had significant UTI and most of the isolates (98.8%) were sensitivity to Imipenem. UTI testing should be conducted periodically, and those who are infected should be treated with antibiotics like Imipenem to avoid complications. New antimicrobials and therapeutic agents with great efficacy, no side effects, ease of availability, and low cost should also be created. In order to reduce the risk of antimicrobial resistance, antibiotic medication should be started following a laboratory culture and sensitivity report. This would not only aid in the prudent use of antibiotics, but it would also help to limit the spread of antimicrobial resistant strains in the research region and the general community.

*Keywords: Uropathogens; bacteria; antibiogram; students; Nigeria; urinary tract infection.*

## 1. INTRODUCTION

When a considerable number of germs, usually larger than 105 cells per millilitre of urine, are discovered in correctly collected mid-stream "clean catch" urine or from a catheter specimen, clinical infection of the urinary tract is considered to exist [1]. Urine is formed in the kidneys through a filtration of blood. The urine is subsequently transported through the urethra and deposited in the bladder. Urine is transferred from the bladder to the outside of the body through the urethra during urination [2].

The presence of bacteria in urine is described as bacteriuria. Bacteriuria accompanied by symptoms are a urinary tract infection, while that without symptoms is known as asymptomatic bacteriuria. Diagnosis is by urinalysis or urine culture. *Escherichia coli* is the most common bacterium found in urinary tract infection [3].

A bacteriuria with urinary symptoms is referred to as a urinary tract infection (UTI). It is one of the most commonly encountered bacterial infections in clinical practise, especially in underdeveloped nations where morbidity and financial costs are considerable. Poor personal hygiene and urinary system abnormalities have been identified as some of the primary variables that predispose to urinary tract infection [4]. The bacteria that cause urinary tract infections differ from location to place, as do their drug sensitivity and resistance patterns. Different microbial pathogens are responsible for UTIs. The most common pathogenic organisms of UTI are *Escherichia coli*, *Staphylococcus saprophyticus*, *S. aureus*, *Proteus sp*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Enterococci* [5].

Frequent and/or painful urination, a desire to urinate despite an empty bladder, fever, and flank pain are all symptoms of UTIs. Urine may include pus and/or seem bloody at times. In pregnant women, UTI is linked to pyelonephritis, premature birth, and miscarriage, and in paediatric patients, it is linked to reduced renal function and end-stage renal disease [6]. Antibiotic resistance in the treatment of urinary tract infections (UTIs) and other bacterial diseases is a major public health issue, particularly in poor nations. In these nations, irrational and indiscriminate antibiotic usage, as well as fake and substandard medications, including antibiotics, are frequent [7,8].

Given these factors, as well as the resulting tendency for bacterial profile changes, doctors should be aware of the degree of susceptibility and resistance of these uropathogens to specific antibiotics in order to treat infections effectively and minimise antibiotic misuse.The essence of this study is to determine the bacteria load of the urine of students in Faculty of Health Sciences, Imo State University, Owerri. Though many people have researched on related topics and few have been able to report the bacteriological load of urine in Imo State University, Owerri without looking into the antimicrobial activity of the isolated organisms, so this work will help to determine the antimicrobial activity of the isolated organisms. Again, most people in our contemporary society are not fully aware of the involvement of certain bacteria in urine and consequently have abused drugs in the course of trying to treat this bacterial disease. Thus, this work was geared towards determining the prevalence rate of urinary tract infections, the bacteria load, and also to add to the already existing knowledge on urinary tract infection among students.

## 2. MATERIALS AND METHODS

### 2.1 Study Population

Urine samples were collected from both male and female students of faculty of health science within the age range of 18 to 40 years. Those students who were on antibiotic treatment prior to the sampling period and those that refused consent were excluded from the study. The total number of students that participated in the study is fifty students (50).

### 2.2 Sample Collection

After thorough cleansing of the external genitalia, the students were taught how to collect clean catch midstream urine samples with wide mouthed sterile screw capped containers. The students provided a total of fifty (50) clean catch midstream urine samples. The urine samples were tagged and delivered to the lab for analysis very away [9].

### 2.3 Cultivation of Samples

The urine samples were cultured using pour plate method (1.0ml) on Nutrient agar (for total heterotrophic aerobic bacteria count), MacConkey agar (for *Enterobacteriacea* family) and Mannitol Salt Agar (For *Staphylococcus species*). Inoculated plates were mixed by rotatory movement, allowed to solidify and inverted at $37^0$c aerobically for 24 hrs.

### 2.4 Enumeration of Bacterial Growth and Isolation

At the end of incubation period, the total heterotrophic aerobic bacterial colonies were enumerated, and then subcultured on fresh sterile medium for further identification. Colonies were counted using electric colony counter. A bacterial count of Hundred and five (105) CFU/mL was considered significant for urinary tract infection (UTI) and counts of Hundred and two(102 – 104) CFU/mL were considered as suspected bacteriuria, while counts less than 102 CFU/ml were considered as non-significant bacterial growth [9].

### 2.5 Identification of the Isolates

Bacterial isolates were identified on the basis of morphological and biochemical characteristics, Gram-staining and motility test were performed. For biochemical characteristics, carbohydrate fermentation, IMViC (indole, Methyl Red, Voges-roskauer, citrate), Oxidase, Catalase, Nitrate, Mannitol Salt Agar (MSA) tests were performed.

### 2.6 Antibiotic Sensitivity Testing

Antibiotic sensitivity testing was done using the disc diffusion method. By plating out, the test organism was seeded on Mueller Hinton agar. The antibiotic sensitivity disc was placed on the surface of the medium with a sterile forceps. The antibiotic disks (Oxoid Ltd. Basingstoke, Hampshire, England) comprised amoxycillin (10 µg), cephradine (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (10 µg), meropenem (10 µg), sulphamethoxazole /trimethoprim (co- trimoxazole) (25 µg), gentamicin (10 µg), netilmycin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), nitrofurantoin (300 µg), amikacin (30 µg), and chloramphenicol (30 µg). The set-up was incubated aerobically at 37°C for 24 hours. The inhibition zone diameters were measured using meter rule after 24 hours incubation and recorded in millimeter (mm).

### 2.7 Statistical Analysis

The data obtained from this study were analyzed statistically using frequency distribution tables and sample percentages and results were represented graphically using pie charts and bar charts.

## 3. RESULTS

Table 1 after culture and isolation of the samples, four groups of isolates were obtained and classified based on their varying colonial morphology as enumerated below. As seen in rows 1, 3 and 4 were non-motile whereas the isolate in row 2 was motile.

Table 3 Shows the bacteriological analysis of the urine samples according to the department of the students. The table shows the urine samples collected from the student in nutrition and dietetics and optometry were most significant for UTI with 60% and 60% respectively, next is Med. Lab. Sci. with 50% followed by Nursing with 40% significance and public health with the least significance 30%.

Table 4 Shows the bacteriological analysis of the urine samples according to the prevalence of the isolates. Isolates 4 showed greater prevalence 14 followed by isolate 3 (13) with isolate 2 (7) and the least is isolate 1 (1).

**Table 1. Colonial morphologies and microscopic evaluation of the bacteria isolates**

| | Colonial Morphology | Motility |
|---|---|---|
| Isolate I | Smooth colonies and pale or colourless | Non motile |
| Isolate II | Slightly pointed ends, polysaccharide capsule, mucoid on maccontrey, lactose fermenting (pink coloured) colonies, slightly raised and translucent with swarming growth and characteristic fishy odour | motile |
| Isolate III | Small, round, smooth glittering yellow colonies | Non motile |
| Isolate IV | Large colonies, thick greyish white, moist smooth, opaque | Non motile |

**Table 2. shows the gram reaction and biochemical characteristics of various isolates, all isolates except isolate 3, were gram negative and rod shape, isolate 3 was gram positive and cocci in form, all having varying colony morphology on the culture plate**

| | Gram reaction | morphology | catalase | coagulase | indole | Methyl red | Vogues proskeur | Citrate utilization | urease | nitrate | oxidase | Identified Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolate I | - | rod | + | - | - | - | + | - | + | + | | *Pseudomonas aeruginosa* |
| Isolate 2 | - | rod | + | - | - | + | - | + | + | + | | *Proteus mirabilis* |
| Isolate 3 | + | Cocci in clusters | + | + | - | + | - | + | + | + | - | *Staphylococcus aureus* |
| Isolate 4 | - | rod | + | - | + | + | - | - | - | + | - | *Escherichia.coli* |

*Key: + = positive*
*- = negative*

**Table 3. Bacteriological significance of the urine sample according to the Departments**

| Department | Number of samples | Significant (%) | Suspected (%) | Non-significant |
|---|---|---|---|---|
| Med. Lab. Sci. | 10 | 5(50) | 1(10) | 4(40) |
| NTD | 10 | 6(60) | 1(10) | 3(30) |
| OPT | 10 | 6(60) | 0(0) | 4(40) |
| Nursing | 10 | 4(40) | 0(0) | 6(60) |
| Public health | 10 | 3(30) | 0(0) | 7(70) |

**Table 4. Shows the Prevalence of the isolated bacterial organisms**

| Organisms | Number of occurrence | Percentage % |
|---|---|---|
| *Pseudomonas aeruginosa* | 1 | 2.9 |
| *Proteus mirabilis* | 7 | 20 |
| *Staphylococcus aureus* | 13 | 37.1 |
| *Escherichia.coli* | 14 | 40 |
| 4 | 35 | 100% |

Fig.1. shows a pie chart showing the departmental base prevalence of the pathogens among the department.

From the pie chart 7, 6, 5, 6 and 3 represents the prevalence of the pathogens in the department

Fig. 2 shows a bar chart with the zone of inhibition (mm) of the antibiotics to the isolated organism plotted against the antibiotics. From the bar chart *Staphylococcus aureus* was found to be sensitive to Imipenem, Ciprofloxacin, Gentamicin, Amitracin Rifampicin, Azithromycin,

Ampicillin, Meropem and resistance to Norfloxacin, tetracycline and erythromycin. *Escherichia coli* was found to be sensitive to imipenem, meropenem Gentamycin, Rifampicin, ciprofloxacin, Amitracin and Azithromycin and resistance to Norfloxacin, tetracycline, ampicillin and erythromycin while *Proteus mirabilis* was sensitive to Rifampicin, ciprofloxacin, amitracin imipenem, azithromycin, Ampicillin and meropenem and resistance to Norfloxacin, tetracycline and Erythromycin while *Pseudomonas* was sensitive to imipenem and rifampicin and resistance to ciprofloxacin, Norfloxacin, Gentamicin, amitracin tetracycline, Erythromycin Azithrodmycin, ampicillin and moropenem.

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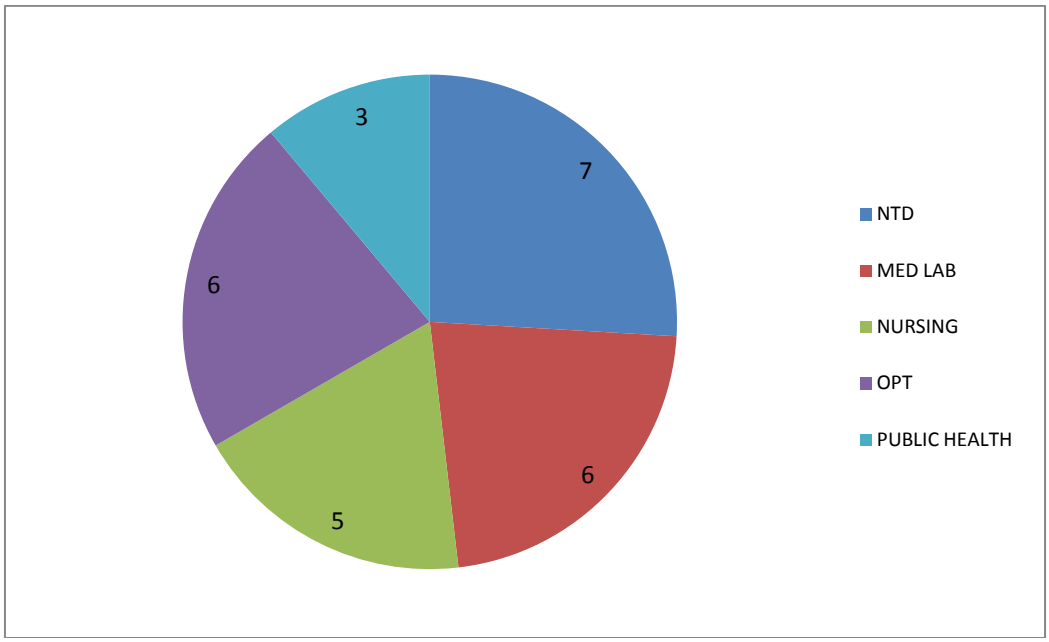


**Fig. 1. A pie chart showing the departmental base prevalence of the pathogens**

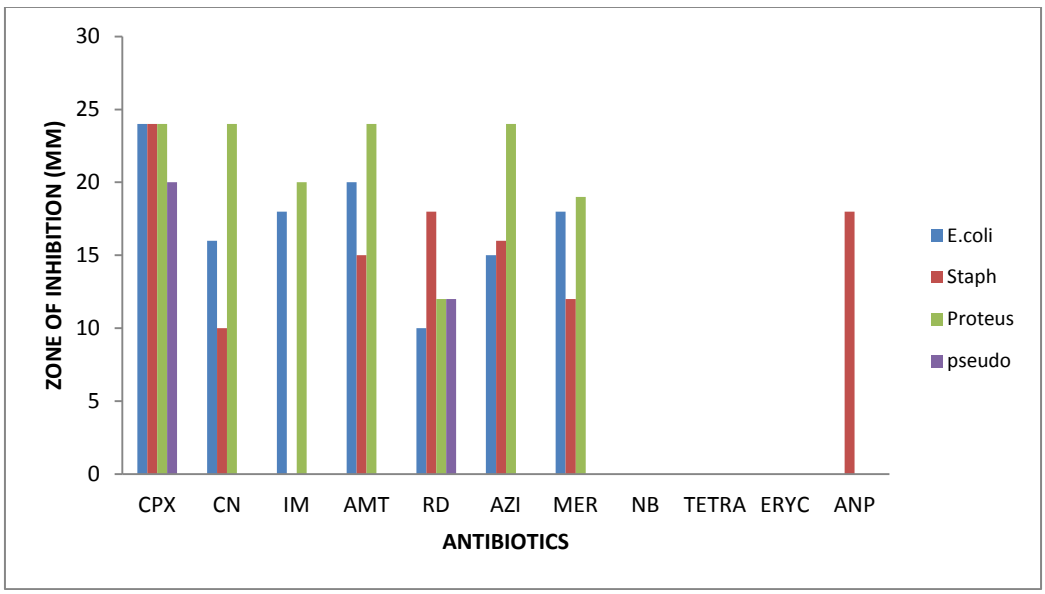


**Fig. 2. A bar chart with the zone of inhibition (mm) of the antibiotics against to the isolated pathogens**

*KEY: CPX= Ciprofloxacin, IM= Imipenem, CN=Gentamycin, AMT= Amitracin, RD= Rifampicin, AZI= Azitromycin, MER= Meropenen, NB= Norfloxacin, TETRA= Tetracycline, ERYC= Erythromycin, AMP= Ampicilin*

**Table 5. Results of antimicrobial susceptibility test**

| NO | Antimicrobial agents test | Concentration Ug/disc | Antimicrobial class | No of resistant to antibiotic |
|---|---|---|---|---|
| 1 | Chloramphenicol | 10 | Phenicols | 48(57.8%) |
| 2 | Ampicillin | 25 | Penicillins | 83(100%) |
| 3 | Nalidixic acid | 30 | Quinolones | 66(79.5%) |
| 4 | Tobramycin | 10 | aminoglycosides | 67(80.7%) |
| 5 | Amitracin | 10 | aminoglycosides | 27(32.5%) |
| 6 | Tetracycline | 10 | Tetracyclides | 65(78.3%) |
| 7 | Ciprofloxacin | 10 | Fluoroquinolones | 45(54.2%) |
| 8 | Imipenem | 10 | Carbapenemes | 1(1.2%) |
| 9 | Cefotaxime | 10 | Cephalosporins | 75(90.3%) |
| 10 | Gentamicin | 10 | aminoglycosides | 32(38.5%) |
| 11 | Meropenem | 10 | carbapenemes | 35(42.1%) |
| 12 | Rifampicin | 5 | Anasamycins | 83(100%) |
| 13 | Azithromycin | 15 | MLSK | 45(54.2%) |
| 14 | Erythromycin | 15 | MLSK | 83(100%) |
| 15 | Nitrofurantoin | 100 | Nitrofurans | 56(67.4%) |
| 16 | Norfloxacin | 10 | fluoroquinolones | 45(54.2%) |

## 4. DISCUSSION

Urinary tract infections (UTIs) are a dangerous infection that is spreading over the world, especially as the incidence of antimicrobial resistance among urinary pathogens rises due to a lack of medicines in use [10]. In this study, the bacteria contents of urine and their antibiogram was investigated among 50 student of the Faculty of Health Science, Imo State University Owerri. Table 4 displays the frequency of microorganisms recovered from urine samples. The finding shows that *Eschericha coli* (40%) was the predominant organism isolated from the urine samples. Followed by *S. aureus,* (37.1%), *P. mirabilis* (20%), while *P. aeruginosa* (2.9%) was the least. This agrees with the report of Bint and Hill, [11]; Boelritwetan et al., [12]; Obirikwurang et al*.,* [9]; Geofrey et al*.,* [13], Poonam and ultra, [14]. The highest occurrence of *E.coli* in the urine samples is in line with the report of Poonam and ultra [14]; Ojo and Anibijuwon, [15]; Boelritwetan et al*.,* [12].

In general, out of the fifty (50) urine samples investigated, 33 samples were significant for UTI ($10^5$CFU/ML), 2 Samples were suspected for UTI ($10^2$-$10^4$CFU/ML), while 15 samples were not significant for UTI (below$10^2$CFU/ML) This can be attributed largely to poor sanitary conditions of their environment due to over congestion of their hostels and agrees with the report of Obirikwurang et al*.,* [9].

Table 3 shows the bacteriological examination of urine samples depending on the students' departments. Samples collected from students in NTD and OPT were significant for UTI (60%) and (60%) respectively, whereas medical laboratory science ranked second in significance (50%), followed by nursing (40%) and the department of public health showed the least significance (30%).

The result of the susceptibility tests in Table 5 shows that the bacterial isolates were highly resistance to Erythromycin. These results are consistent with the report Chowdhury et al., [16]. Overall, the findings of this investigation revealed high levels of harmful bacteria in urine samples. The antibiotic imipenem was the most effective in suppressing the bacterial isolates. The clinical consequences of these findings are significant. As a result, these antibiotics are most effective in the treatment of urinary tract infections. This however, contrasts the findings of Geofrey et al*.,* [13] in which the isolated gram-positive and gram-negative bacteria isolated from urine, also showed 100% sensitivity towards Amitracin.

## 5. CONCLUSION

The present study showed that the urine samples collected from students of Faculty of Health Science, Imo State University, Owerri showed significant UTI of which the cases were most among students of the Department of Nutrition and Dietetics. This study also examined the effect of sixteen conventional antibiotics on different bacteria isolated from urinary tract infections. The results indicated the dominance *of E. coli* isolates with a percentage of (40%),

followed by *Staphylococcus aereus* with a percentage of (37.1%). All bacterial isolates were resistant to Erythromycin, Norfloxacin, Tetracycline. Most of the isolates (98.8%) were sensitive to Imipenem. Based on the results of this study a periodic testing for UTI is advocated and those found significant should be advice to go for treatment with antibiotics like Imipenem to avoid complications. Furthermore, it is now critical to develop new antimicrobial and therapeutic agents that are highly effective, have no side effects, are easy to obtain, and are less expensive. With the rise of antimicrobial resistance in mind, it is strongly recommended that antibiotic therapy be started only after a laboratory culture and sensitivity report. This would not only aid in the prudent use of antibiotics, but it would also help to prevent the spread of antimicrobial resistance strains among the research population and the general public.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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