Original Research Article

ASSESSMENT OF BACTERIOLOGICAL AND ANTIBIOGRAM OF UROPATHOGENS AMONG STUDENTS IN FACULTY OF HEALTH SCIENCES, IMO STATE UNIVERSITY, OWERRI.

ABSTRACT

The Assessment of bacteriological and antibiogram of uropathogens among students in 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 MacConkey agar and CLED (Cystine Lactose Electrolyte deficient) using streak method and were incubated at 37^{o}C for 24 hrs. 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 plates were then sub cultured for further identification. The 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 10^2CFU/ml). Based on departmental studies, the subjects from the Department of Nutrition and Diatetics and 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. Periodic testing for UTI is therefore advocated and those found to be infected need to be treated with antibiotics like Imipenem to avoid complications. Also, it is now very necessary to develop new antimicrobials and therapeutic agents having high effectiveness with no side effects, easy availability and less expensive. So by keeping the emerging antimicrobial resistance in mind, it is strongly inferred that the antibiotic therapy should only be commenced after culture and sensitivity report from the laboratory. This would not only help in the sensible use of antibiotics, but also would restrain the spread of antimicrobial resistant strains in the study area and the community at large.

Keywords: Uropathogens, Bacteria, Antibiogram, Students, Nigeria, Urinary Tract Infection

1.0 INTRODUCTION

Clinical infection of the urinary tract is said to exist where a significant number of microorganisms, usually greater than 10^5 cells per milliliter of urine are detected in properly collected mid-stream "clean catch" urine or from a catheter specimen [1]. Urine is formed in the kidneys through a filtration of blood. The urine is then passed through the urethra to the bladder, where it is stored. During urination the urine is passed from the bladder through the urethra to the outside of the body [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].

Urinary tract infection (UTI) is described as a bacteriuria with urinary symptoms. It is one of the most common bacterial found infections seen in clinical practice, particularly in developing countries with a high rate of morbidity and financial cost. Some of the key factors predisposing to urinary tract infection have been attributed to poor personal hygiene and urinary tract abnormalities [4]. The causative agents of urinary tract infection vary from place to place and the also vary in their susceptibility and resistance patterns to antibiotics. UTIs are caused by different microbial pathogens. The most common pathogenic organisms of UTI are *Escherichia coli*, *Staphylococcus saprophyticus*, *S.aureus*, *Proteus sp*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Enterococci* [5].

Symptoms of UTIs include frequent and/or painful urination, a feeling to urinate despite having an empty bladder, fever and flank pain. At times, the urine may contain pus and/or appear bloody. UTI is a risk factor for pyelonephritis, preterm delivery and miscarriage

among pregnant women, and is associated with impaired renal function and end-stage renal disease among pediatric patients [6].

Antibiotic resistance in the treatment of UTI and other bacterial infections constitute a major public health problem, especially in the developing countries. Irrational and indiscriminate use of antibiotics as well as fake and substandard drugs, including antibiotics is common in these countries [7, 8].

In view of these and attendant tendency for changes in bacteriological profile, it is worthwhile that the degree of susceptibility and resistance of these uropathogens to various antibiotics be known to clinicians for effective treatment of infections they cause and to avoid 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 educating the students about urinary tract infection by determining the bacteria load, and also to add to the already existing knowledge about urinary tract infection among students.

2.0 MATERIALS AND METHODS

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).

Sample collection

The students were properly educated on how to collect clean catch midstream urine samples with wide mouthed sterile screw capped containers after proper cleaning of the external genitalia. A total of fifty (50) clean catch midstream urine samples were collected from the students. The urine samples were labeled and immediately sent to the laboratory for analysis [9].

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 37^0c aerobically for 24 hrs.

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].

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.

Antibiotic Sensitivity Testing

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

Statistical Analysis

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

3.0 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 the table, 1,3 and 4 were non motile whereas isolate 2 were motile.

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**	**coagullase**	**indole**	**Methyl red**	**Voguesprobsk**	**Citrate utilization**	**urease**		**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: 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 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 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 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%

Figure 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

Figure 1: A pie chart showing the departmental base prevalence of the pathogens

Figure 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.

Figure 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%)

4.0 DISCUSSION

Urinary tract infections (UTIs) are serious infection worldwide especially with the prevalence of antimicrobial resistance among urinary pathogens increasing worldwide due to absence of antibiotics in practice [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. The frequency of bacteria isolated from the urine samples is presented in Table 4. The result show 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 (10^5CFU/ML), 2 Samples were suspected for UTI (10^2-10^4CFU/ML), while 15 samples were not significant for UTI (below10^2CFU/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].

Bacteriological analysis of the urine samples based on the department of the students was shown in table 3. 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 susceptibility tests as shown in table 7. Shows that Erythromycin were highly resistance to the bacteria isolates and have varying resistance levels to most antibiotics. These results are consistent with the report Chowdhury *et al*., [16]. Overall, the results of this study indicated significant pathogenic bacterial counts in the urine samples. Imipenem was the most effective antibiotic in inhibiting the bacterial isolates. These results have important clinical implications. Thus, these antibiotics do best in case 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.

Conclusion

The present study has observed 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 aeurus* 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. Also, it is now very necessary to develop new antimicrobial and therapeutic agents having high effectiveness with no side

effects, easy availability and less expensive and by keeping the emerging antimicrobial resistance in mind, it is strongly suggested that the antibiotic therapy should only be Commenced after culture and sensitivity report from the laboratory. This would not only help in the sensible use of antibiotics, but also restrain the spreading of antimicrobial resistance strains among the study population and the community at large.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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