Antibiotics Susceptibility Testing Of Urinary Pathogens Isolated From Hospital Attendees In Ile-Ife, OSUN State.

¹Adekunle O.C., ² Gbadeyan O. F., ³ Sanusi T.O., ⁴.Ojedele R.O

^{1,2,4} Department of Medical Microbiology and Parasitology and ³Department of Community Medicine of Ladoke Akintola University of Technology, Osogbo, Nigeria.

Corresponding Author: Adekunle O.C

Abstract: Urinary tract infection (UTI) is one of the most prevalent infections and of a great health burden both in Nigeria and in the world at large. This study was carried out to determine the common uropathogens, establishing antibiotics susceptibility profile of the urinary pathogensand identify the hospital department with highest UTIs in tertiary hospital in Ile-Ife, Osun State. Urine samples were collected from all the participants who had urinary problems from ranging from ages 1-120years. Mid Stream Urine samples were collected for urinalysis and later cultured. Disc diffusion method of sensitivity testing was used and the susceptibility pattern was interpreted. Three hundred urine samples were tested which were collected from different departments. One hundred and sixty (160) 53.3% were females while one hundred and forty 140 (46.7%) were males respectively. Gram negative isolates had a prevalence of 261 (87%) while Gram positive isolates had 39 (13%).

The bacteria isolated were Klebsiella species 134(44.7%), Escherichia coli 89(29.7%), Staphylococcus aureus, 39 (13%), Pseudomonas species 28 (9.3%), Proteus species 5 (1.7%), Coliforms 4 (1.3%) and Citrobacter species 1(0.3%). Outpatient department had the highest number of bacterial isolates 142 (47.3%) while the intensive care unit had the least isolates 5(1.7%) and 13 (4.3%) their wards were not indicated. The highest susceptibility for the gram negative was Ciprofloxacin, Ofloxacin and Nitrofuraton while Imipenem and Cefixime show the highest susceptibility among the tested antibiotics for Gram positive bacteria.

Keywords: Urinary pathogens, antibiotics, urine samples, hospital departments

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I. Introduction

Urinary tract infections are among the most commonly encountered infections, both in the commonly and in hospitals. UTIs may be community acquired or nosocomial community acquired infection (Bahadin, 2011). UTI is classified into uncomplicated and complicated infections with respect to choice, for treatment (Sherifa *et al.*, 2012).

Urinary tract infection is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the digestive tract which enters through the opening of the urethra and multiplies to cause infection (Mwaka, 2011).

The urethra and urinary bladder are the most frequent sites of infections within the urinary tract (Akoachere *et al.*,2012) It was found that women were more prone to UTIs than men with the risk of infection related to the frequency of sex (Nicolle, 2008).

Urinary tract infections may involve only the lower urinary tract or both the upper and the lower tracts.

Urinary tract infection can be either symptomatic or asymptomatic. Patients with significant bacteriuria who have symptoms referable to the urinary tract are said to have symptomatic bacteriuria (Smelov *et al.*, 2016). Asymptomatic is a condition characterized by presence of bacteria in two consecutive clear voided urine and in a patient without classical symptoms.

Antimicrobial chemotherapy especially antibiotics has played an extremely important role in reducing the morbidity rates of human infectious diseases (Trevino *et al.*, 2015). However, the extreme use of antimicrobial agents has resulted in the emergence of resistant strains of bacteria. (Fasugba *et al.*, 2015).

Antibiotics resistance is a growing problem, some may be due to overuse of antibiotics in human but some probably due to the use of antibiotic as growth promoters in food of animals (Johnson *et al.*, 2006).

Today resistance has become a large and growing problem in infection treatment and that account for most of Africa's disease burden, including respiratory and diarrheal disease (Okenke *et al.*, 2007). Resistance equally compromises the management of urinary diseases, sexually transmitted diseases or diseases spread by the faecal-oral route, such as typhoid fever, dysentery and other diarrheal diseases (Okenke *et al.*, 2007, Okonko *et al.*, 2009b).

The aim of this study was to investigate the antibiotics susceptibility profile of urinary pathogens isolated from tertiary hospital attendees in Ile-Ife, Osun State.

II. Materials And Method

Ethics statement

This work was performed according to University ethics committee code of conduct.

Study area

The study was carried out at Obafemi Awolowo Teaching Hospital Ile-Ife Complex (O.A.U.T.H.C), Ile-Ife, Osun State, Nigeria. The study was a retrospective study and was conducted in October, 2018.

Collection of samples

Clean samples of mid stream urine were collected from all participants. The first part of the urine was voided out and the middle part of the urine was passed into a 4-5ml sterile universal bottle and the later part voided out. The samples were labelled and transported to the laboratory for immediate processing within one hour after collection for aerobic bacterial culture, but where a delay of more than 1-2 hours was unavoidable; the sample was refrigerated at 4°C.

Macroscopy examination of urine

When urine samples were brought for bacteriological examinations, the samples were examined for their physical properties such as volume, colour and appearance and reported. The colonial appearance (colour, shape, size, odour, and consistency) of the organisms was noted after 24 hours of incubation at 37°C. Urine samples were examined microscopically as a wet preparation to detect significant dysuria, (pus cells in urine) red cells, casts, yeasts cells, bacteria.

Culturing of urine specimen

A sterile wire loop was used to inoculate a loopful of well mixed urine on a cysteine lactose electrolyte-deficient (CLED) agar. It was incubated aerobically at 35-37°C

Initially strains were identified based on the morphological behaviour of the isolates on various differential media. All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 minutes at 151 b pressure. Examination of the cultures and the appearance of urinary pathogens on CLED agar were noted.

Biochemical tests such as catalase, coagulate test, urease test, citrate test, oxidase test, motility test, indole test and sugar fermentation test. Moreover, sensitivity testing was carried out on all isolates using agar diffusion disc method using diagnostic sensitivity agar.

Discrete colonies of all isolates were inoculated into sterile peptone water in order to give a concentration of 10 bacteria per ml. Commercially prepared antibiotic paper discs were placed on the plates and incubated aerobically at 37°C for 24 hours following the overnight incubation and the plates were examined for the zone of inhibition.

The diameter of the zone of inhibition produced by each antibiotic disc was measured. The result was interpreted as susceptibility or resistance to the antibiotic agent depending on the length of zone of inhibition produced compared to the reported standard length. The resistance strains grew to the edge of the disc.

III. Results

A total number of three hundred (300) patients were examined. One hundred and forty (140) were males while one hundred and sixty (160) were females. The study showed that the prevalence of urinary tract infections was higher among young and middle aged patients 31-40years while age 91-100 years and 101-110years had the least prevalence of infection as shown in Table 1.

In this study, females had the highest number of bacterial isolates when compared with males as shown in Table 2.The most common isolates in this study were the Gram negative bacilli which amount to sixty one percent of all the total isolates. In the Gram negative bacilli, the predominant isolate was the *Klebsiella species* 134(44.7%) as shown in Table 3, followed by *Escherichia species* 89(29.7%) while the *Citrobacter species* was 1(0.3%). In the gram positive bacteria, the main organism identified was *Staphylococcus aureus* 39 (13%). Table 4 recorded the number of bacterial isolates found in different age groups. Age group 31-40 had the highest number 63 and age group 91-100 and 101-110 had the lowest number 1 isolate in each group.

The distribution of all the isolates by wards was recorded in Table 5, the highest isolates came from outpatient department 142(47.3%) followed by accident and emergency 18(6.0%) while intensive care unit 5(1.7%) had the least number of isolates. The antimicrobial susceptibility and resistance pattern of the isolated pathogens showed that among the tested antibiotics, for Gram negative bacteria Ceftriazone had the highest

susceptibility followed by Cefixine, Imipenem, Ciprofloxacin, Ofloxacin, Nitrofuratoin, Ceftazidine, Gentamycin, Augmentin and Ampicillin while Imipenem and Cefixime show the highest susceptibility among the tested antibiotics for Gram positive isolates in Table 6.

Klebsiella species which was the highest isolates gave high susceptibility to Ceftriazone 118 (88.1%) and have least susceptibility to Ampicillin 71(53.0%). Staphylococcus aureus, the only Gram positive bacteria isolated shows highest susceptibility to Imipenem and Cefixime 35(89.7%). It was discovered that the isolates was completely resistance to Streptomycin 39(100%).

TABLE:1 AGE DISTRIBUTION OF SUBJECTS AS WELL AS PERCENTAGES OF BACTERIAL ISOLATES BACTERIAL ISOLATES (%)

| AGE GROUPS (Years) | Staphylococcus aureus NO | Klebs iella species NO | Escherichia col NO | i <i>Proteus species</i> NO | Pseudomonas species NO | Citrobacter species NO | Coliform organisms NO | TOT | AL |
|-----------------------|-----------------------------|---------------------------|-----------------------|--------------------------------|---------------------------|---------------------------|--------------------------|-----|------|
| 1-10 | 0 | 5 | 5 | 0 | 0 | 0 | 0 | 10 | 3.3 |
| 11-20 | 3 | 3 | 5 | 0 | 0 | 0 | 1 | 12 | 4.0 |
| 21-30 | 7 | 18 | 24 | 0 | 0 | 0 | 0 | 49 | 16.3 |
| 31-40 | 14 | 26 | 13 | 0 | 8 | 0 | 2 | 63 | 21.0 |
| 41-50 | 0 | 18 | 9 | 1 | 3 | 0 | 1 | 32 | 10.0 |
| 51-60 | 7 | 21 | 9 | 4 | 1 | 0 | 0 | 42 | 14.0 |
| 61-70 | 4 | 23 | 10 | 0 | 8 | 0 | 0 | 45 | 15.0 |
| 71-80 | 2 | 13 | 9 | 0 | 6 | 1 | 0 | 31 | 10.3 |
| 81-90 | 1 | 4 | 4 | 0 | 0 | 0 | 0 | 09 | 3.0 |
| 91-100 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 01 | 0.3 |
| 101-110 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 01 | 0.3 |
| 11112-0 | 1 | 2 | 0 | 0 | 2 | 0 | 0 | 05 | 1.7 |
| TOTAL | 39 | 134 | 89 | 5 | 28 | 1 | 4 | 300 | 100 |

TABLE 2: PREVALENCE OF URINARY ISOLATES AND SEX DISTRIBUTION OF PATIENTS

| TOTAL | 39 | 134 | 89 | 5 | 28 | 1 | 4 | | 300 | 100.0 |
|--------------------------|------------------------|------------------|-----------------|------------------------|------------------------|------------------|----|-------|------------|-------|
| MALE FEMALE | 19 20 | 66 68 | 38 51 | 4 | 14 14 | 0 | 3 | | 140 160 | 53.3 |
| SEX | NO | NO NO | NO 20 | NO | NO | NO 1 | NO | | 140 | 467 |
| Staphylococcus aureus | Klebs iella species | Escherichia coli | Proteus species | Pseudomonas species | Citrobacter species | Coliforn species | | TOTAL | % | |
| BACTERIAL ISOI | ATES (%) | | | | | | | | | |

TABLE 3: DISTRIBUTION OF BACTERIAL ISOLATES IN THE URINE SAMPLES

| ISOLATES | NUMBER | 9% | |
|-----------------------|--------|------|--|
| Staphylococcus aureus | 39 | 13 | |
| Klebsiella species | 134 | 44.7 | |
| Escherichia coli | 89 | 29.7 | |
| Proteus species | 5 | 1.7 | |
| Pseudomonas species | 28 | 9.3 | |
| Citrobacter species | 1 | 0.3 | |
| Coliform organisms | 4 | 1.3 | |
| TOTAL | 300 | 100 | |

TABLE 4: NUMBER OF ISOLATES IN DIFFERENT AGE GROUPS

| AGE GROUP | NO OF ISOLATES | PERCENTAGE[%] |
|-----------|----------------|---------------|
| 1-10 | 10 | 3.3 |
| 11-20 | 12 | 4.0 |
| 21-30 | 49 | 16.3 |
| 31-40 | 63 | 21.0 |
| 41-50 | 32 | 10.7 |
| 51-60 | 42 | 14.0 |
| 61-70 | 45 | 15.0 |
| 71-80 | 31 | 10.3 |
| 81-90 | 9 | 3.0 |
| 91-100 | 1 | 0.3 |
| 100-110 | 1 | 0.3 |
| 111-120 | 5 | 1 |
| TOTAL | 300 | 100 |

TABLE 5: URINARY PATHOGENS FROM DIFFERENT WARDS AND CLINICS

| | *** | TTT 07717 4 077 (A/) |
|----------------------------|-----|----------------------|
| WARDS | NO | PERCENTAGES (%) |
| OF ISOLATES | | |
| OPD (MOP, SOP) | 142 | 47.3 |
| ACCIDENT AND EMERGENCY | 18 | 6.0 |
| PEDIATRICS | 16 | 5.3 |
| ICU | 5 | 1.7 |
| MEDICAL (MALE AND FEMALE) | 18 | 6.0 |
| SURGICAL (MALE AND FEMALE) | 6 | 2.0 |
| OBSTETRICS & GYNAECOLOGY | 37 | 12.3 |
| RENAL | 6 | 2.0 |
| CLINICS | 39 | 13.0 |
| NOT INDICATED | 13 | 4.3 |
| TOTAL | 300 | 100 |
| KEY | | |

OPD - OUT PATIENT DEPARTMENT

MOP-MEDICAL OUT PATIENT

SOP - SURGICAL OUT PATIENT

ICU-

INTENSIVE CARE UNIT

TABLE 6: ANTIBIOTICS SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM URINE SAMPLE

| ORGANISMS | | AUG | CAZ | GEN | OFL | AMP | NIT | CPR | IMI | CXM | CTR | STR | CXC | ERY |
|----------------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | NO |
| Klebsiella species S | | 19 | 57 | 43 | 79 | 71 | 19 | 96 | 91 | 98 | 118 | NA | NA | NA |
| | R | 120 | 77 | 91 | 55 | 63 | 120 | 38 | 43 | 36 | 16 | NA | NA | NA |
| Escherichia coli | S | 40 | 54 | 65 | 59 | 11 | 54 | 68 | 79 | 69 | 73 | NA | NA | NA |
| | R | 49 | 35 | 54 | 30 | 78 | 35 | 11 | 20 | 20 | 16 | NA | NA | NA |
| Proteus species | S | NA | 3 | 2 | 4 | NA | NA | 3 | 4 | 3 | 3 | NA | NA | NA |
| | R | NA | 2 | 3 | 1 | NA | NA | 2 | 1 | 2 | 2 | NA | NA | NA |
| Pseudomonas | S | NA | 17 | 16 | 19 | NA | NA | 21 | 16 | 25 | 24 | NA | NA | NA |
| | R | NA | 11 | 12 | 9 | NA | NA | 7 | 12 | 3 | 4 | NA | NA | NA |
| Citrobacter spe | ciesS | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | NA | NA | NA |
| | R | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | NA | NA | NA |
| Coliform organismsS | | 2 | 3 | 3 | 3 | 1 | 2 | 3 | 3 | 3 | 2 | NA | NA | NA |
| | R | 2 | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 1 | 2 | NA | NA | NA |
| Staphylococcus | S | 9 | 5 | 11 | 16 | 1 | NA | 14 | 35 | 35 | 26 | 0 | 2 | 13 |
| aureus | R | 30 | 34 | 28 | 23 | 38 | NA | 25 | 4 | 4 | 13 | 39 | 37 | 26 |

KEY: R-Resistant S-Susceptible

GEN-GENTAMYCIN OFL-OFLOXACIN

AMP-AMPICILIN

CPR-CIPROFLOXACIN CXM-CEFIXIME

STR-STREPTOMYCIN CXC-

CLOXACILLIN

CTR-CEFTRIAZONE IMI-IMIPENEM AUG-AUGMETIN APLLICABLE

CAZ-CEFTACIDINE NIT-NITROFURATOIN ERY-ERYRHROMYCINNA-NOT

IV. Discussion

Urinary tract infection ranks one of most common medical disease encountered in medical practise with significant morbidity and health costs occurring from neonate to elderly (Kabugo et al., 2016). The prevalence of urinary tract infections was higher among young and middle age patients, this was also consistent with Andabati, 2010. In this study, females were affected more than the males, this finding was in agreement with other studies from many parts of the world showing a statistically predominance of females ((Mwaka,

2011)) which may be due to close proximity of the female urethra to the anus and shorter urethra. Also, lack of bacteriostatic properties of prostatic secretions have been reported as factors that influence the higher prevalence in female.

In this study, *Klebsiella species* were the most predominant bacteria isolated, this is in agreement with Nordmann *et al*;(2009), who found out that urinary tract infection is an important nosocomial infection in hospital patients and in patients with repeated attacks on those who received previous antibiotics. According to Kabugo *et al.*, 2016, the commonest uropathogen isolated was *Escherichia coli* at 50%, this was followed by *Staphylococcus aureus* at 15.4%, but from this study the commonest pathogen was *Klebsiella* (44.7%)followed by *Escherichia coli* (29.7%). Age group 31-40 years had the highest number unlike Akoachere *et al.*, 2012 which recorded age group 29-30 which highest number. Also, from the study that outpatient department had the highest isolates of 142(47.3%) while intensive care unit 5(1.7%) has the least number of isolates

Staphylococcus aureus, the only Gram positive bacteria isolated shows highest susceptibility to Imipenem and Cefixime 35(89.7%). It was discovered the isolate was completely resistance to Streptomycin (100%) and ampicillin also have a very bad susceptibility pattern. This can be due to the misuse of these common antibiotics by the patients (Mulugeta, 2014).

In vitro, *Klebsiella species* which was the predominant isolate gave high susceptibility to Ceftriazone 118(88.1%) and Cefixime 98(73.1%) respectively. *Escherichia coli*, the second most isolated organism also show high susceptibility to Ceftriazone 73(82.0%) and Imipenem 79(88.8%) respectively.

Other isolates shows a good susceptibility pattern to Imipenem, Nitrofuratoin, Ofloxacin, Ceftazidine and Augmentin. Data presented in this study indicated that some of the commonly antibiotics used in urinary tract infections against Gram negative organisms are still effective but regular monitoring should be in place in order to make reliable information available for optimal empirical therapy for patients with Urinary Tract Infections(UTIs)(Zalamanovici *et.al.*,2010).

V. Conclusion

Conclusively, the most common Gram negative organism isolated from urinary tract was *Klebsiella species* and the effective antimicrobial agents used were Ceftriazone, Cefixime and Imipenem while *Staphylococcus aureus*, the only Gram positive cocci isolated showed high susceptibility to Imipenem and was completely resistance to Streptomycin.

VI. Recommendations

The community should be advised on the importance of personal hygiene and preventive measures against urinary tract infection.

Empirical treatment of urinary tract infection without culture and sensitivity results should be discouraged.

There should be effective campaign against indiscriminate use of antibiotics to inhibit the proliferation of resistant strains of bacteria.

There is need for continuous monitoring of bacteria antibiotics susceptibility before prescription in order to ensure adequate treatment of urinary tract infections.

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