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Prevalence and Antibiotic Resistance Patterns of Bacterial Isolates from Urine Samples of Patients Attending Kwara State Civil Service Hospital Ilorin, Nigeria.

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Abstract

Antibiotic resistance has become alarming throughout the world and most especially in third world countries in particular. Human urine samples(n= 80) of patients attending the Kwara state Civil Service Hospital Ilorin, North Central Nigeria were analysed for Urinary Tract Infections(UTIs) and Antibiotic Susceptibility pattern of isolated organisms. The urine samples were cultured on blood agar, MacConkey agar and Cysteine lactose electrolyte deficient (CLED) agar while antimicrobial susceptibility test was determined using Kirby-Bauer disc diffusion method. The following bacteria were isolated *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus epidermidis, Enterococcus faecalis* and *Klebsiella pneumoniae.* The isolates comprise of 57.1% Gram negative bacteria and 42.9% Gram positive bacteria. This study revealed that antibiotics such as Ciprofloxacin and Ofloxacin(100% and 71.4% susceptibility respectively) are the most effective against the isolates, the antibiotic resistance pattern of the isolates indicates emerging multi drug resistance among urinary tract bacterial pathogens.

Keywords: UTI**,** Antimicrobial susceptibility, disc diffusion, uropathogen, *E. coli.*

Introduction

Urinary tract infections (UTI) represent one of the most common bacterial infections in the urinary system. Most of these infections involve the lower urinary tract, namely the bladder and urethra (Abejew *et al*., 2014). It is the second most common infectious presentation in community. UTIs have become one of the most common disease encounters in clinical practices. There is an estimation that over 150 million UTIs cases occur worldwide annually

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(Gobernado *et al*., 2007). The clinical significance of the infections is due to its high mortality rate, malignant and chronic hypertensions and the chronic renal failures followed by chronic pyelonephritis (Sharifian *et al*., 2006; Zhanel *et al*., 2006). The severity of UTI depends on the virulence of bacteria and susceptibility of the host (Jadhov, 2011).

This infection varies in patients with different gender, age, and presence of associated genitourinary abnormalities (Nicolle, 2002). Extensive antibiotic prescription is related to a higher prevalence of antibiotic resistant bacteria (Cohen-Nahum *et al*., 2010). The elderly are prescribed antibiotics more frequently than younger adults, and antibiotic courses are common at nursing homes (Deneman *et al*., 2011; Fagan *et al*., 2012). When bacteria gets to the bladder or kidney and multiply in urine, they may cause a urinary tract infection. The symptoms include frequent feeling and / or need to urinate, pain during urination and cloudy urine. The risk factors for UTIs include sexual activity, gender, genetics and presence of urinary catheters. Women are found to be highly susceptible to UTIs than men and the reason is that their urethra is much shorter and much closer to the anus than in males. Females do not have the bacteriostatic properties of prostatic secretions (Nicolle, 2008). The structure of the female's urethra and vagina also makes it susceptible to trauma during sexual intercourse as well as bacteria being massaged up the urethra and into the bladder during pregnancy and or child birth (Kolawole *et al*., 2009).

UTI is the most common infectious diseases among women (Hooton et al., 2004). Nearly one woman out of three, will have at least one episode of UTI requiring antimicrobial therapy by the age of 24 years, and almost 50% of all women will experience at least one episode of UTI during their lifetime (Karlowsky *et al*., 2001). UTIs are also the most common infections in hospital and extended-care settings, it has been reported that 2% – 3% of admitted patients are suffering from UTI (Mazzulli, 2002; Chomarat, 2000). This is associated with a high risk of morbidity, mortality, extra financial budget and even, fatal consequences particularly in children (Hasan *et al*., 2007). UTIs are mainly caused by Gram negative rods (GNR) which account for 80 to 85% and the leading causative organisms are *Escherichia coli* (75 to 95%) and *Klebsiella pneumoniae, Enterobacter* species, *Proteus* species and gram-positive bacteria like *Enterococcus* species, and *Staphylococcus saprophyticus. E. coli* is the most common organism causing both community as well as hospital acquired UTI (Sobel and Kaye, 2010; Tanvir *et al*., 2012).

The etiology of UTIs and the antibiotic susceptibility of urinary pathogens, both in community and hospitals, have been changing over the past years and recently, the antibiotic resistance has become a major global problem (Hooton *et al*., 2004). Much attention has not been paid to the etiological role of urogenital tract infection (UTI) and the attendant consequences on sperm functions/quality in recent years. There are many reasons for this problem, including antibiotic consumption while feeding the animal, inappropriate prescription of antibiotics and poor infection control strategies (Castro-Orozco *et al*., 2010). As a result of constant variation of isolated UTI causative microorganisms and antibiotic sensitivity pattern, this research tends to investigate the antibiotic resistance pattern among selected patients attending Kwara State Civil Service Hospital, Ilorin, Nigeria.

2. Materials and Methods

Sample collection

Freshly voided early morning mid-stream urine samples of eighty patients attending Civil Service hospital, Ilorin were aseptically collected into sterile specimen bottles. They were transported to the laboratory in ice pack for immediate analysis. This research was carried out between November 2012 and February 2013. The age range of the patients was also taken into consideration.

Total Bacterial count

The bacterial load of the samples was determined by the method according to Santo *et al*. (2007). The samples were serially diluted after enrichment in buffered peptone water. Appropriate dilution was transferred unto plate count agar using spread plate technique at 37 ^{o}C for 24hr, after which the count was determine using colony counter.

Bacteriological Analysis

Appropriate dilution of well mixed urine samples were inoculated into Blood agar, MacConkey agar and Cysteine Lactose electrolyte deficient agar (CLED). The inoculum was well streaked out to ensure distinct colony with wire loop according to Chessborough, 2006. The culture plates were incubated at 37^{o}C for 24 - 48hr. The isolates were identified using the conventional Standard methods (Cowan and Steel, 1974; Chessborough, 2006).

Antibiotic Susceptibility Testing.

The antibiotic sensitivity test was carried out on Mueller-Hinton agar by the method of Kirby-Bauer disc diffusion test as recommended by Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2010). Molten Mueller-Hinton agar were poured into plates and allowed to set at room temperature. Colonies of isolated bacteria were thereafter streaked on the solidified agar surface. Antibiotic disc were then placed on the streaked agar surfaces using sterile forcep. The plates were then incubated at 37^{o}C for 24hr. The zones of growth inhibition were measured with ruler. The antibiotics used include;

Table 1: List of antibiotics used and their concentration.

Antibiotic	**Concentration**
Amoxycillin	30µg,
Ofloxacin	10µg,
Streptomycin	30µg,
Chloramphenicol	30µg,
Ceftriazone	10µg,
Gentamycin	10µg,
Cotrimoxazole	25µg,
Ciprofloxacin	5µg,
Erythromycin	5µg,
Augumentin	30 µg,

3. Results and Discussion

Urinary tract Infections (UTIs) are one the most commonly prevalent infection. This study describes the susceptibility and resistance profile of urine isolates. Bacterial load from this study ranges from 1.1 x 10^2 to 3.4 x 10^6. A total of eighty samples of urine were collected for this study from patients at the Kwara State Civil Service Hospital, Ilorin. The bacterial load was considerably high which is in agreement with findings of Abubakah (2009) who reported

similar result. Although bacterial count of less than 10^5ml should not be considered less or insignificant because true infection may be found with a count as low as 130cfu/ml. From all the samples eighty samples analysed, 106 different strains were isolated and identified using the conventional methods in which Gram negative Rod (GNR) has the highest prevalence of 86.6% with Gram positive Cocci(GPC) being 13.2%. The identified isolates includes, *Escherichia Coli* 47(44.3%), *Klebsiella pneumonia*e 19(17.9%), *Staphylococcus aureus* 7(6.6%), *Stapylococcus epidermidis* 5(4.7%), *Pseudomonas aeruginosa*15 (14.2%), *Enterococcus feacalis* 2 (1.9%) and *Proteus mirabilis* 11 (10.4%) as reported in Table 2.

Table 2: Frequency of occurrence of Isolates from Urine Samples

Organism	**n%, n=106**
E. coli	47(44.3)
K. pneumoniae	19(17.9)
P. aeruginosa	15(14.2)
P. mirabilis	11(10.4)
S. aureus	7(6.6)
S. epidermidis	5(4.7)
E. feacalis	2(1.9)

The distribution of isolate based on the age group of patients was shown in Table 3. The highest occurrence of the isolate was recorded in age group 20 – 29 years while the lowest range was recorded in age group 70^+. Also lower level of isolates was recorded in age range 0-9 and 60- 69years.

Table 3: Distribution of isolates based on age group.

Age (yr)	***S. aureus***	***P. mirabilis***	***K. pneumoniae***	***S. epidermidis***	***E. coli***	***P. aeruginosa***	***E. faecalis***
0-9	-	-	1	-	1	-	-
10-19	1	1	1	1	5	1	-
20-29	4	5	9	2	19	9	1
30-39	1	2	4	1	12	2	-
40-49	1	1	1	1	5	2	1
50-59	-	1	1	-	3	1	-
60-69	-	1	1	-	1	-	-
70^{+}	-	-	1	-	1	-	-

Antimicrobial susceptibility test was shown in Table 4. All isolates were susceptible to Ciprofloxacin while five isolates were susceptible to Oflaxacin. The isolates were resistant to the commonly available antibiotics such as Tetracyclin, Ampicillin, Augmentin, Chloramphenicol and Erythromycin.

Table 4: Antimicrobial susceptibility test on isolates.

Antibiotic	Isolate						
	S. aureus	***P. mirabilis***	***E. coli***	***S. epidermidis***	***K. pneumonia***	***P. aeruginosa***	***E. faecalis***
Amoxillin	-	-	19(S)	-	-	-	-
Ofloxacin	21 (S)	15 (S)	18(S)	19 (S)	11 (I)	14 (I)	15 (S)
Streptomycin	-	-	-	-	-	-	-
Chloramphenicol	-	-	-	-	-	-	7
Ceftriazone	-	-	-	-	-	-	-
Gentamycin	9	-	13 (I)	15 (S)	-	-	-
Pefloxacin	24 (S)	8	12 (I)	11 (I)	13 (I)	-	22 (S)
Cotrimoxazole	-	-	10	-	-	-	-
Ciprofloxacin	19 (S)	16 (S)	21(S)	17 (S)	18 (S)	17 (S)	16 (S)
Erythromycin	-	-	-	-	-	-	6 (R)
Augumentin	-	7	-	-	6	-	-
Tetracycline	-	-	14(I)	-	-	10	-
Ampicillin	-	-	-	4	-	-	-

KEY: I : INTERMEDIATE, S : SENSITIVE, - : NO ZONE OF INHIBITION

Prevalence rate of UTIs among selected patient was shown in Figure 1. Significant growth rate of 62.5% was achieved while there are no significant growths in 25.5%. Isolated bacteria in this study were predominantly Gram-negative organisms which are similar to the reports of Tessema *et al*. (2007). Enterobactericeae family are more prevalent with *Escherichia coli* being the highest which agrees with the findings of Russell *et al.* (2007) and Castro- Orozco *et al*. (2010). *E. coli* predominate at 44.3% which is similar to the findings of Prakash and Saxena (2013), although the findings of some researcher shows higher percentage (El-Astal, (2005); Shaikh *et al.*, 2005) who reported above 60%. The prevalence of *E. coli* differs in some other findings in which *Pseudomonas aeruginosa* recorded highest prevalence followed by *Klebsiella* spp. (Aboderin *et al.*, 2009). According to this study, *Klebsiella* spp. was the second most common UTI causing bacteria as also reported by Abubakar, (2009)

which correlates with our study. *E.coli* has been found to be responsible for 90% of first incidence of UTIs in healthy individuals and most nosocomial UTIs (Lewis, 2010).

Some studies recorded UTI in babies and elderly (Vaezzadeh and Sharifi-Yazdi, 2001; Sharifian *et al*., 2006) but this reports was contrary to our findings. Similar result was recorded by the findings of Mirzarazi *et al.* (2013). Other bacteria isolated includes *Klebsiella pneumoniae* (17.9%), *Pseudomonas aeruginosa* (14.2%), *Proteus mirabilis* (10.4%), *Staphylococcus aureus* (6.6%), *Staphylococcus epidermidis* (4.7%) and *Enterococcus faecalis* (1.9%) which were in support of the research work of Alemu *et al.* (2012) and Majumder *et al.* (2014).

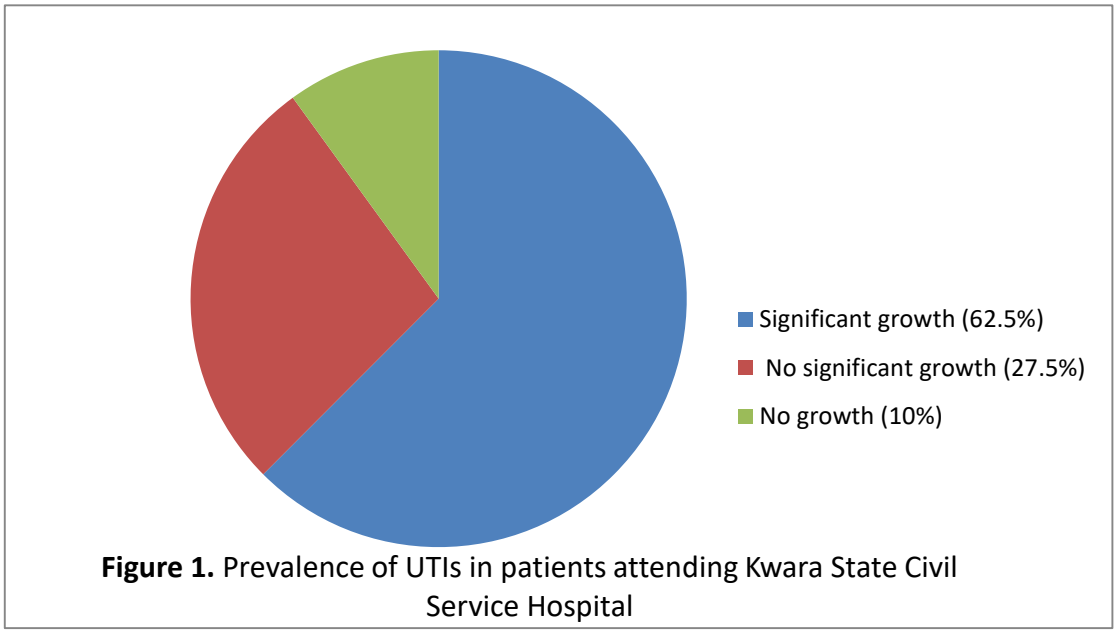

Figure 1. Prevalence of UTIs in patients attending Kwara State Civil Service Hospital

In this study, the incidence of UTI was found prevalent in patients within age group 20-29years followed by age group 30-39years which support the finding of Prakash and Saxena (2013). This might be as a result of sexual activeness of the member of this age group. The lower records of case of UTI in children and elderly may be as a result awareness for improved hygiene. According to the result of susceptibility test, Ciprofloxacin was found to be 100% effective against all the isolates while Ofloxacin has 71.4% susceptibility. The findings of Oluremi *et al*. (2011) show the two antibiotics being susceptible at 66.7% and 46.7% respectively. The presence of *S. aureus* being a normal skin floral could also be related

to the fact that it gets into the tissues, urine especially when the individual's immune system is weakened, and then causes an infection as an opportunistic pathogen (Shankel, 2007).

Table 5: Multiple antibiotic resistance pattern of isolates

Isolates	**Antibiotics**	**Percentage Resistance**
Staphylococcus aureus	AMX, STR, CHL, GEN, CEF, COT, TET, ERY, AUG, AMP	10(76.9%)
E.coli	CHL, STR, CEF, E, AUG,AMP,COT	7(53.8%)
Proteus mirabilis	AMX, STR, CHL, CEF, GEN, PEF, COT, ERY, AUG, TET, AMP,	11(84.6%)
Klebsiella pneumonia	STR, AMX, CHL, CEF, ERY, AUG, TET, AMP, OFL, GEN, COT	11(84.6%)
Staphylococcus epidermidis	CHL, AMP, TET, AUG, ERY, STR, AMX, CEF, COT	9(69.2%)
Pseudomonas aeruginosa	PEF, AMP, CHL, AUG, ERY, AMX, STR, TET, GEN, COT, CEF	12(92.3%)
Enterococcus faecalis	GEN, AUG, AMP, CEF, ERY, AMX, STR, TET, COT, CHL	10(76.9%)

KEY:- AMX: Amoxillin, PEF: Pefloxacin, GEN: Gentamycin, CEF: Ceftriazone
AUG: Augumentin, TET: Tetracycline, AMP: Ampicillin, ERY: Erythromycin
COT: Cotrimoxazole, CHL: Chloramphenicol, STR: Streptomycin, OFL: Ofloxacin

The result of multiple resistances to antibiotics is shown in Table 5. The high resistance to the commonly used antibiotics like Ampicillin and Tetracycline, Amoxillin and Streptomycin by this research has raised serious concerns as it was in agreement with findings researchers (Sahm *et al.,* 2001; Rabasa *et al*., 2009; Festo *et al*., 2011Okunola *et al*., 2012; Msaki *et al*., 2012). These antibiotics are widely available at the primary health care level, antibiotics are accessed from local chemists without prescriptions, resulting in irrational use of the drugs. The long period of usage and under dosage causes the organisms to develop resistance. Zhanel *et al.* (2006) and Akram *et al.* (2007) reported that antibiotic resistance is a major

clinical problem in treatment of infection caused by these organisms and the resistance has been found to increase from year to year and it also varies from country to country. Age, sex and geographical location can also contribute to amount of antibiotic resistance of urinary isolates. The highest multiple resistance was recorded in *Pseudomonas aeruginosa* (92.3%), followed by *Klebsiella pneumonia* and *Proteus mirabilis* (84.6%). The least resistance was recorded in *Escherichia coli* (53.8%). All the results agree with study by Abubakah, (2009).

4. Conclusion

In conclusion, the isolation of bacterial uropathogens with a higher multi drug resistance rates for commonly used antibiotics calls for effective monitoring of bacterial antibiotics susceptibility before prescription in order to achieve complete treatment of urinary tract infections and also to prevent the spread of multi drug resistant strains. Comparison among different studies concerning resistance of uropathogens to different commonly used antibiotics should be considered in different periods. This comparism must consider the limitation of resistance to antimicrobial which vary from country to country. This study revealed that bacterial resistance in uropathogens continues to be a great problem. Proper treatment of UTIs should be advocated, after culture and sensitivity have been carried out.

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