Antibiotics Sensitivity and Resistance Patterns of Uropathogens to Quinolones in Patients with Urinary Tract Infections in a Nigerian Community.

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ABSTRACT

Urinary tract infection is one of the most common bacterial infections in humans. Urine samples from one hundred patients within the age range of 20 to 50 years were collected and examined for the presence of urinary tract infections. The significant growth was observed in 9% of the population. A majority of the isolates in this study were totally sensitive to levofloxacin, and showed various degrees of sensitivity to nalidixic acid, ciprofloxacin and ofloxacin; while some of the isolates were totally resistant to cotrimoxazole and amoxicillin.

(Keywords: urinary tract infections, UTI, urine, patients, isolates, sensitive, antibiotic resistant)

INTRODUCTION

Urinary Tract Infection (UTI) is among the most common bacterial infection in humans both in the community and hospital settings. It occurs in all age groups and in both genders and usually requires urgent medical treatment (Omoregie *et al*., 2008). Urinary tract infection is a broad term that encompasses both the asymptomatic microbial colonization and symptomatic infection with invasion and inflammation of the urinary tract structures (Fluit *et al*., 2000; Santo *et al*., 2007). It is a common type of bacterial infection occurring both in the community and hospital settings, including acute and long care hospital patients (El-Astal, 2004; Mordi and Erah, 2006).

The most common cause of UTI is Gram-negative bacteria that belong to the family *Enterobacteriaceae*. Members of this family include *Escherichia coli, Klebsiella, Enterobacter,* and *Proteus.* Also the Gram-positive *Staphylococcus saprophyticus* plays a role, especially among young women (Kunin, 1997). An estimated 130–175 million cases of UTI occur annually worldwide with huge social costs, and evaluation and management of UTI approximates US$1.6 billion (Foxman, 2002). The figures can be higher if costs of self-diagnosed and self-medicated infections are taken into consideration (Hooton and Stamm, 1997; Gupta and Hooton, 2004). UTI accounts for as many as 35% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients (Weinstein, 1997).

The quinolone class of antibiotics is widely used to treat urinary tract infections with encouraging results (Khaki *et al*., 2009). Alongside aminoglycosides gentamicin (10µg), neomycin, streptomycin (30µg), the quinolones and the fluoro-quinolones groups of antibiotics ofloxacin (5µg), ciprofloxacin (10µg), are routinely used by fertility specialists to treat such bacterial infections occurring prior to the *in vitro* fertilization treatment or when high concentration of leukocytes are present in the semen of these patients irrespective of microbial evidence of infection (Khaki *et al*., 2008).

Fluoro-quinolones are often considered agents of first choice in patients with UTI. Ciprofloxacin, levofloxacin and norfloxacin are increasingly being used, even in uncomplicated UTI (Hooton, 2003a). However, increasing fluoro-quinolone resistance is a serious public health threat, and it is essential that the indiscriminate use of these agents is avoided, if we are to preserve the efficacy of these drugs to critical diseases. For example, the prevalence of fluoro-quinolone resistance in uropathogenic *Escherichia coli* (UPEC) causing uncomplicated UTI is very high in some parts of the world, especially in Southern Europe, where it has been reported to be as high as 20% (Kahlmeter, 2003).

It has been shown that the susceptibility of uropathogens to the quinolone groups of antibiotics has not been extensively studied in Nigeria. However, there have been some reports on the prevalence of pathogenic microorganisms and their susceptibility to other groups of antibiotics (Kolawole *et al.*, 2009).

This study was conducted to determine the microbial aetiology of urinary tract infections among patients in four different hospitals in Ota, Ogun State and their antimicrobial susceptibility pattern to some quinolone class of antibiotics.

MATERIALS AND METHODS

Study Population

The study population was drawn from patients attending four different hospitals in Ota, Ogun State. One hundred (100) patients reporting for urinary tract infections made up of 50 males and 50 females aged between 20-50 years were involved in this study.

Exclusion Criteria

Those that were excluded from this study included pregnant women and patients on antibiotic therapy.

Sample Collection

Early morning midstream urine (MSU) specimens were obtained from the patients. The patients were taught on how to collect early morning midstream urine and also the patients supplied the following information on the specimen's bottle: age and sex.

Sample Processing

Prior to culturing, samples were preserved in the refrigerator at 4-6°C and processed within 2 hours of collection. The samples were examined macroscopically. A calibrated sterile platinum wire loop that is 4.0 mm in diameter which is to deliver 0.01 ml of the urine sample onto cystine lactose electrolyte deficient (CLED) agar plate was used. All plates were incubated at 37°C aerobically for 24 hours. The plates were then examined for bacterial growth and isolates were characterised biochemically as described by Cowan and Steel (1993) and Cheesbrough (2000).

Antibiotic Susceptibility Test

This test was carried out to determine the antibiotic susceptibility pattern of different isolates. Nutrient agar plates were inoculated with isolates from stock cultures. The Kirby-Bauer disc-diffusion test which conforms to the recommended standard of the Clinical and Laboratory Standards Institute (CLSI), formerly National Committee for Clinical Laboratory Standards (NCCLS) was used as reported by Bauer and co-workers, 1966.

Turbidity of the inoculum of various isolates of uropathogen is compared with 0.5 McFarland standard and each of the isolates was inoculated onto the surface of sterile nutrient agar plates using a sterile swab in order to ensure even distribution of the inoculum, the plates were allowed to dry for not more than 15 minutes and the antibiotic discs with different concentrations were placed on the surface of the agar plates. After 30 minutes of applying the disc, the plates were inverted and incubated for 24 hours at 37°C. The clear zone that developed around each disc were measured as the zones of inhibition from underneath each plate with the aid of a ruler in centimetre (cm) and converted to millimetre (mm). The antimicrobial discs include the following: Ofloxacin (5µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Nalidixic acid (30µg), Gentamicin (10µg), Nitrofurantoin (200µg), Cotrimoxazole (25µg), Amoxicillin (25µg), and Tetracycline (25µg).

Statistical Analysis

The data obtained were subjected to chi-square test at 5% ($p<0.05$) confidence interval for the male and female patients.

RESULTS

Urine samples from one hundred (100) patients within the age range of 20 to 50 years from four different hospitals in Ota were collected and examined for the presence of urinary tract infections. The color of the urine samples ranged from pale yellow, light yellow, amber to clear or turbid in transparency. From the urinalysis, 29 samples (16 males and 13 females) were positive

to bilirubin which indicates liver abnormality, cancer or hepatitis; 16 samples (9 males and 7 females) were positive to urobilinogen; 9 samples (8 females and 1 male) were positive to protein which indicates what is known as proteinuria; 25 samples (10 females and 15 males) had traces of protein in their urine which indicates a normal urine; 2 samples (2 females) were positive to nitrite which is a rapid method for detecting asymptomatic urinary tract infection; 15 samples (11 females and 4 males) were positive to leucocyte which also indicates a bacterial infection; 8 samples (3 females and 5 males) had blood in their urine which indicates haematuria; 7 samples (5 males and 2 females) had glucose in their urine; 5 samples (3 females and 2 males) had a pH above 8.0 and this also indicates a urinary tract infection.

In this study, significant growth was observed in 9% of the population, while 91% showed insignificant growth. Prevalence of urinary tract infection was higher in females (7%) than in males (2%). The organisms isolated were *Staphylococcus saprophyticus* (35%), *Enterococcus faecalis* (20%), *Pseudomonas aeruginosa* (13%), *Enterobacter* species (12%), *Proteus* species (7%), *Neisseria* species (6%), *Escherichia coli* (5%), *Klebsiella* species (2%). The most prevalent of these organisms is *Staphylococcus saprophyticus* which was found more in women who are sexually active and the least prevalent of the organisms was *Klebsiella* species (see Table 1 and Figures 1, 2, and 3).

Majority of the isolates in this study were totally sensitive to levofloxacin, and showed various degree of sensitivity to nalidixic acid, ciprofloxacin and ofloxacin. While some of the isolates were totally resistant to other non-quinolone class of antibiotics such as cotrimoxazole and amoxicillin (Figures 4, 5, and 6).

Table 1: Distribution of Uropathogens among Patients in all Hospitals.

Uropathogens isolated	Occurrence of Uropathogens in each of the Hospitals												Total
	A			B			C			D			
	M	F	ST	M	F	ST	M	F	ST	M	F	ST	
Staphylococcus saprophyticus	1	1	2	9	12	21	3	5	8	-	4	4	35
Enterococcus faecalis	2	1	3	3	10	13	1	-	1	-	3	3	20
Pseudomonas aeruginosa	3	-	3	6	1	7	1	-	1	-	2	2	13
Enterobacter species	2	2	4	3	3	6	1	-	1	-	1	1	12
Proteus species	3	1	4	2	-	2	-	1	1	-	-	-	7
Neisseria species	-	1	1	2	1	3	1	1	2	-	-	-	6
Escherichia coli	1	1	2	2	1	3	-	-	-	-	-	-	5
Klebsiella species	2	-	2	-	-	-	-	-	-	-	-	-	2
Sub-Total	14	7	21	27	28	55	7	7	14	-	10	10	
Total			**21**			**55**			**14**			**10**	**100**

KEYS: M – Male; F – Female; ST – Sum Total

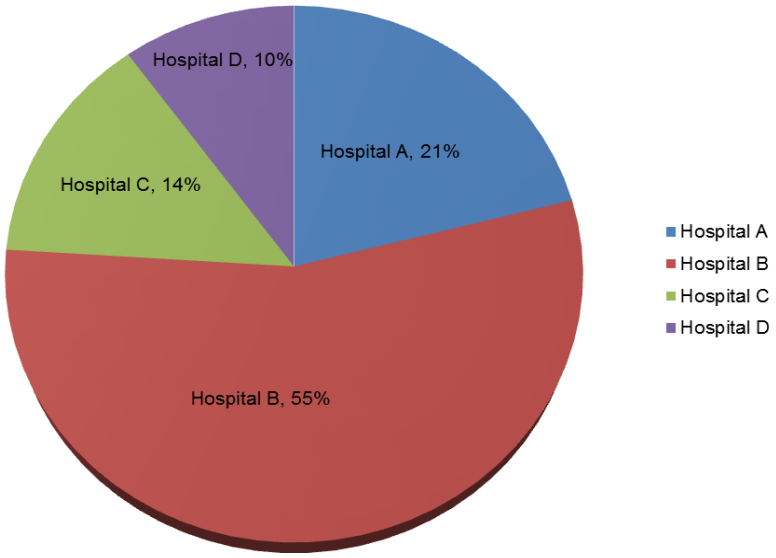


Figure 1: Distribution of the Bacterial Isolates in the Four Hospitals.

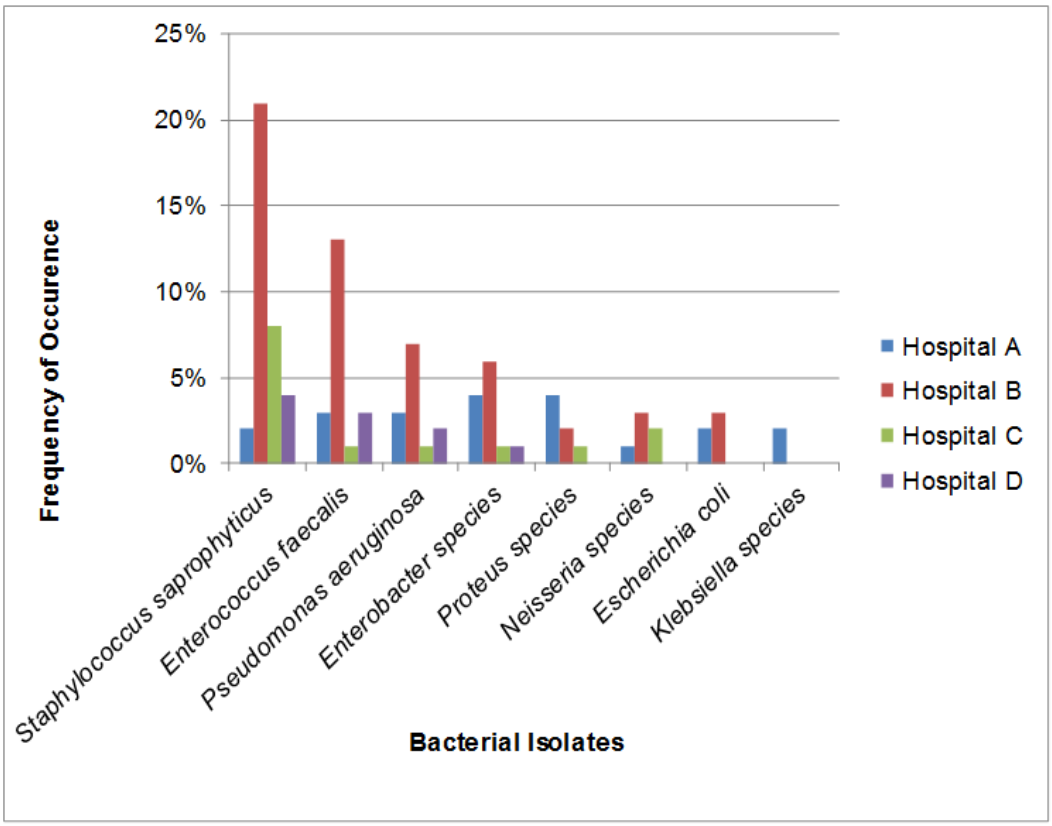


Figure 2: Comparison of the Prevalence of all the Bacterial Isolates in the Four Hospitals

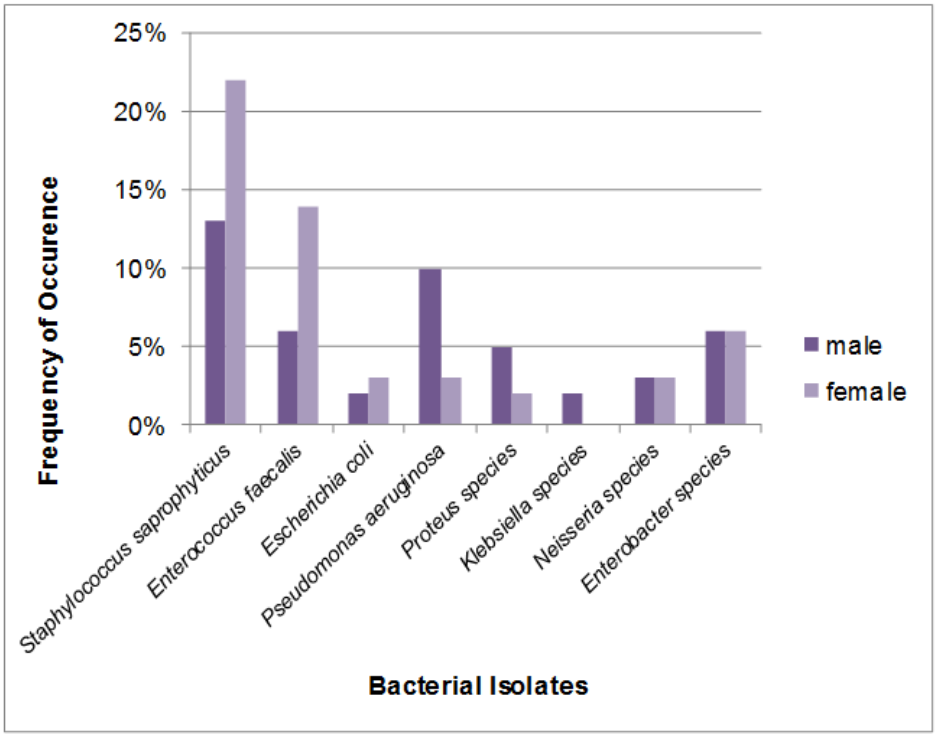
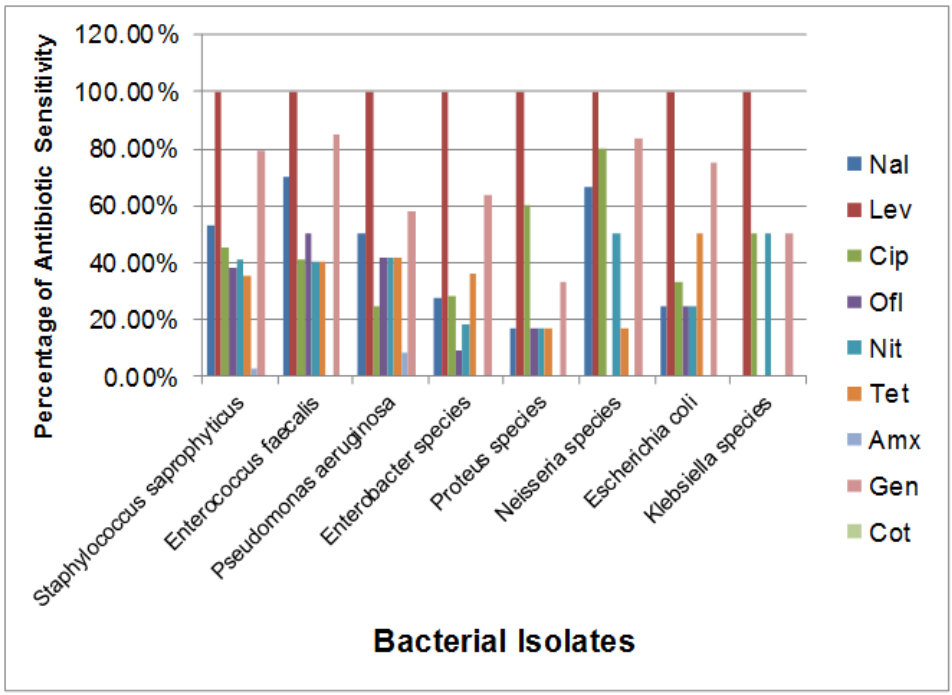
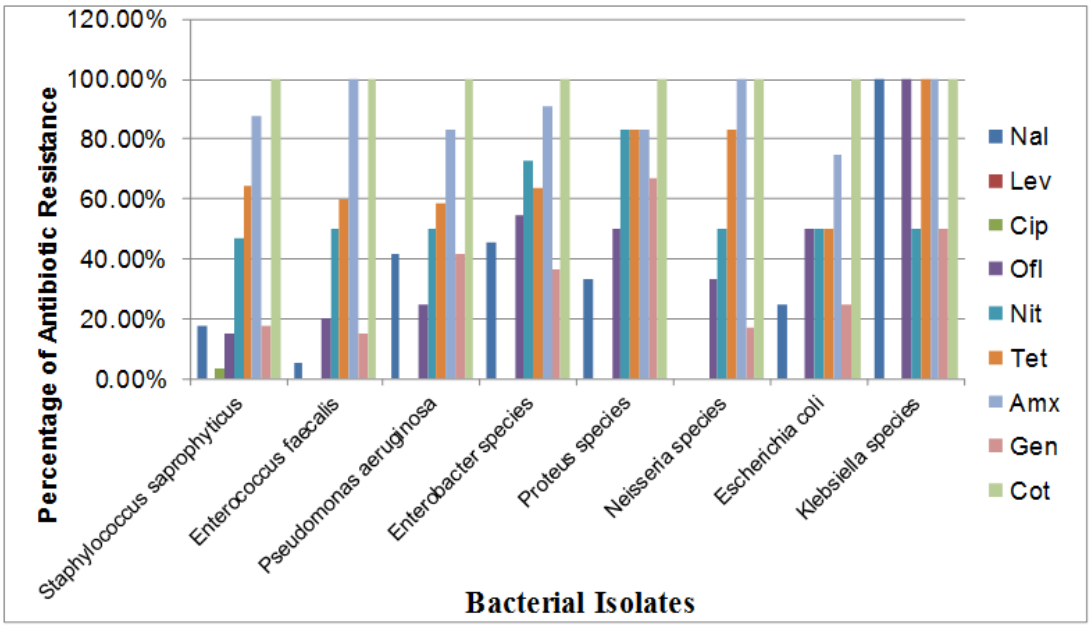


Figure 3: Distribution of Bacterial Isolates among Male and Female Patients.



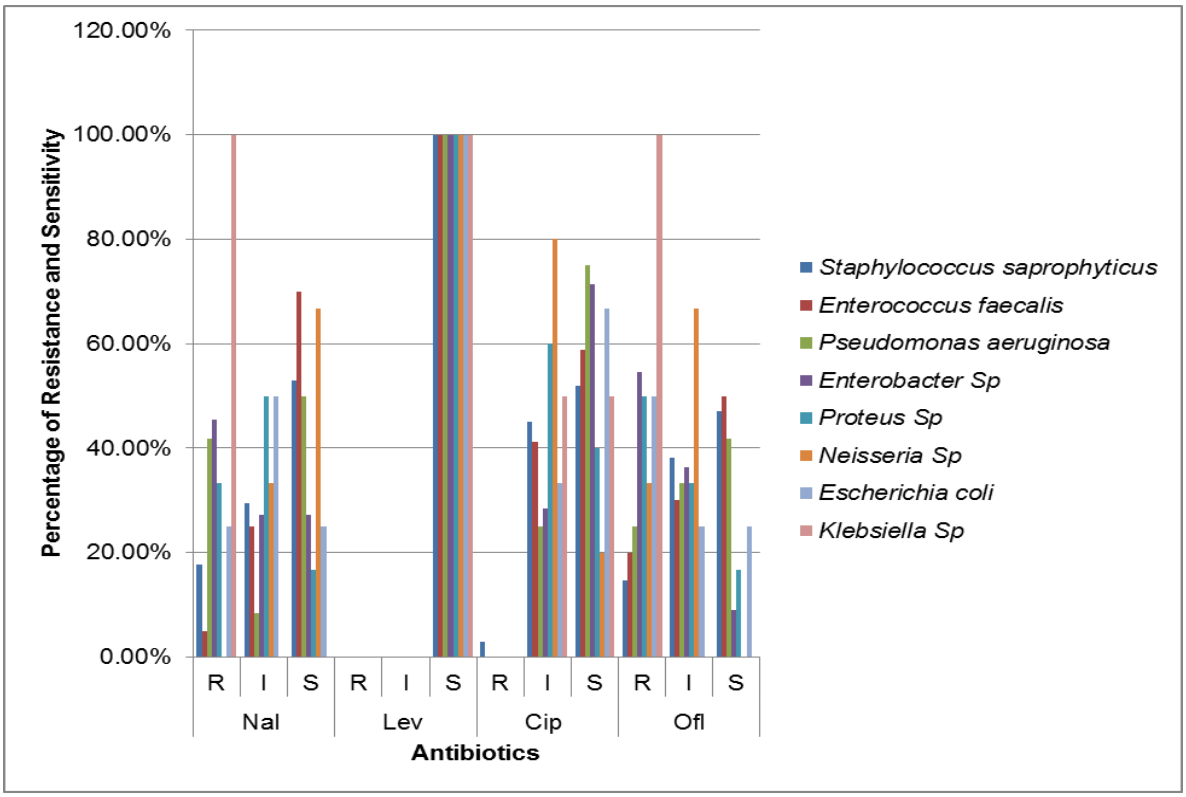
KEYS: Nal – Nalidixic acid; Lev – Levofloxacin; Cip – Ciprofloxacin; Ofl – Ofloxacin; Nit – Nitrofurantoin; Tet – Tetracycline; Amx – Amoxicillin; Gen – Gentamicin; Cot – Cotrimoxazole

Figure 4: Antibiotic Sensitivity Patterns of Uropathogens



KEYS: Nal – Nalidixic acid; Lev – Levofloxacin; Cip – Ciprofloxacin; Ofl – Ofloxacin; Nit – Nitrofurantoin; Tet – Tetracycline; Amx – Amoxicillin; Gen – Gentamicin; Cot – Cotrimoxazole

Figure 5: Antibiotic Resistance Patterns of the Uropathogens



KEYS: R – Resistant; I – Intermediate; S – Susceptible; Nal – Nalidixic acid; Lev – Levofloxacin; Cip – Ciprofloxacin; Ofl – Ofloxacin

Figure 6: Antibiotic Sensitivity and Resistance Patterns of Uropathogens to Quinolones.

DISCUSSION

This study showed that the prevalence of urinary tract infection was observed in 9% of the population. The females had a higher prevalence of (7%) than the males (2%). This observation is in agreement with Nwanze and co-workers (2007) who reported that urinary tract infections are far more frequent in females than in males during youth and adulthood.

Higher prevalence of UTI in females than males has also been observed by other workers (Mordi and Erah, 2006; Mbata, 2007). It has also been reported in several studies that women who are sexually active, and especially if they use contraceptives, foams, gels, diaphragm and spermicides which are known to promote greater colonization of the vagina are at higher risk of developing UTIs (Mbata, 2007; Nwanze *et al*., 2007).

While 91% of the population showed insignificant growth which can be indicated by a mixed bacterial flora and that the urine was collected with care to minimize contamination and examined soon after collection before the commensals would have had time to multiply significantly.

The organisms isolated in this study include *Escherichia coli, Klebsiella* species*, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Proteus* species*, Neisseria* species*.* These bacterial species have also been isolated in other studies of urinary tract infections (Gruneberg, 1994; Kunin, 1994; Akerele *et al*., 2000; Mordi and Erah, 2006; Nwanze *et al*., 2007).

According to Cheesbrough (2000), 10^4 cfu/ml is significant for urinary tract infection. Based on the result of this study,10^4 cfu/ml was used which is in agreement with the earlier works of Arav-Boger and co–workers (1994) which stated that patients with bacterial counts between 10^2 and 10^4cfu/ml has microorganisms typical for urinary tract infection (*Escherichia coli, Staphylococcus saprophyticus* and enteric Gram–negative bacteria).

The most prevalent organism isolated in this study was *Staphylococcus saprophyticus* which accounted for 35 (35%) of the isolates within the age range of 21–50 representing 22 from females and 13 from males and this is in agreement with Cheesbrough (2004) that *S. saprophyticus* is predominantly found among sexually active young women. The second most common isolate in this study was *Enterococcus faecalis* which had been previously reported by Mordi and Erah (2006) as an agent of UTI. This organism accounted for 20% of the bacterial isolates with 14 from females and 6 from males; followed by *Pseudomonas aeruginosa* which accounted for 13% of the isolates, *Enterobacter* species accounted for 12%, *Proteus* species, *Neisseria* species and *Klebsiella* species accounted for 7%, 6% and 2% of the isolates respectively. *Escherichia coli* which is the commonest aetiology agent of UTI in previous studies accounted for only 5% of the isolates in this study and this is in agreement with results from several studies that have shown that the proportion of *E. coli* as a principal causative agent of UTI is slowly declining, being replaced by other members of the *Enterobacteriaceae* and *Enterococci* (Gruneberg, 1994).

The urinalysis in this study showed that 2 samples (2 females) were positive to nitrite which is a rapid detection for urinary tract infection, 15 samples were positive to leukocyte (11 females and 4 males). The presence of nitrite has a predictive value for UTI, since nitrite are formed as a metabolic product from bacteria that breakdown nitrate to nitrite (e.g. *Escherichia coli, Proteus species, Klebsiella species etc*.). If the dipstick is positive to either nitrite or leucocyte, this increases the probability of UTI to about 80%, while dipstick negative in both nitrite and leucocyte reduces the probability of UTI to 20% (Bent *et al*., 2002).

In this study it was discovered that majority of the bacterial isolates were totally sensitive to levofloxacin; while other isolates showed various degrees of sensitivity to nalidixic acid, ciprofloxacin and ofloxacin. The isolates showed total resistance to cotrimoxazole and amoxicillin which are not quinolones. This is similar to other reports where quinolones are the most effective (susceptible) antibiotics (Krumpermann, 1983; Burbige *et al.,* 1984; Ebie *et al.,* 2001; Ehinmidu, 2003; Mbata, 2007). Amoxicillin and cotrimoxazole which are commonly used antibiotics were poorly effective against majority of the organisms isolated in this study. However, these non-quinolone antibiotics were used in this study to compare their effectiveness in treating UTI with quinolone group of antibiotics.

All the quinolones substantially inhibited the growth of the uropathogens to various degrees. Among the Gram-negative bacteria, *Pseudomonas aeruginosa* was susceptible with a profile of 6 (50%) to nalidixic acid, ofloxacin 5 (41.7%), levofloxacin 6 (100%), ciprofloxacin 2 (25%), tetracycline 5 (41.7%), nitrofurantoin 5 (41.7%), gentamicin 7 (58.3%) this data obtained is compared with (Nwanze *et al*., 2007); this study recorded susceptibility rates for *Enterobacter* species with a profile for nalidixic acid 3 (27.3%), levofloxacin 5 (100%), ciprofloxacin 2 (28.5%), ofloxacin 1 (9%), gentamicin 7 (63.6%). This study also recorded susceptibility rate for *Proteus* species which had a susceptibility profile for nalidixic acid 1 (16.67%), levofloxacin 4 (100%), ciprofloxacin 3 (60%), ofloxacin 1 (16.7%), similar to the reports of Rai and co-workers (2008). The susceptibility pattern followed similar trend for the other Gram–negative bacteria isolated in this study.

Among the Gram–positive bacteria, *Staphylococcus saprophyticus* had a susceptibility profile for nalidixic acid 18 (52.9%), levofloxacin 14 (100%), ciprofloxacin 13 (45%), ofloxacin 13 (38.2%), gentamicin 27 (79.4%). *Enterococcus feacalis* had a susceptibility profile for nalidixic acid 14 (70%), levofloxacin 9 (100%), ciprofloxacin 7 (41.1%), ofloxacin 10 (50%), gentamicin 17 (85%). Rai and co-workers (2008) also reported a sensitivity pattern to nalidixic acid (33.3%), ofloxacin (100%) and ciprofloxacin (66.7%).

The resistance patterns of the uropathogens were totally different from the susceptibility patterns because most of the isolates were totally resistant to some of the quinolones. *Staphylococcus saprophyticus* had a resistance profile for nalidixic acid 6 (17.6%), ofloxacin 5 (14.7%). *Enterococcus faecalis* had a resistance profile for nalidixic acid 1 (5%), ofloxacin 4 (20%), for *Pseudomonas aeruginosa* nalidixic acid 5 (41.7%), ofloxacin 3 (25%). The resistance pattern followed similar trend for the other Gram-negative bacteria.

The antibiotic susceptibility profile for all the UTI bacterial isolates in this study was nalidixic acid 47 (49.5%), levofloxacin 44 (100%), ciprofloxacin 33 (43.4%), ofloxacin 31 (32.6%), nitrofurantoin 35 (36.8%), tetracycline 33 (34.7%), amoxicillin 2 (2.1%), gentamicin 68 (71.5%). While the antibiotic resistance profile for all the UTI bacterial isolates in this study was nalidixic acid 22 (23%), ciprofloxacin 1 (76%),ofloxacin 27 (28.4%), nitrofurantoin 51 (53.7%), tetracycline 62 (65.3%), amoxicillin 86 (90.5%), gentamicin 25 (26.3%), cotrimoxazole 95 (100%). All the isolates were highly resistant to older drugs (cotrimoxazole and amoxicillin).Use of aminoglycosides especially when only one type is employed, may lead to an increased level of resistance (Swartz, 1997).

This result of the antimicrobial susceptibility patterns shows that the quinolones used in this study were useful antibiotics for the treatment of UTI because they inhibited most of the uropathogenic bacterial isolates, with levofloxacin having the highest activity among them all, followed by nalidixic acid, ciprofloxacin and ofloxacin. Antibiotic sensitivity and resistance patterns of organism change rapidly over a short period of time, in developing countries where these drugs are purchased over the counter without prescription. Therefore, indiscriminate prescription and use of these antibiotics should be discouraged in both community and hospital settings and adequate enlightenment of the public should be adopted by the authority.

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