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Distribution and Antibiotic Susceptibility Pattern of Bacterial Pathogens Causing Urinary Tract Infection in Mubi General Hospital, Yola-Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study was conducted to determine the distribution and antimicrobial susceptibility of uropathogens among patients attending Mubi general hospital as well as to determine the effect of gender on the etiology of bacterial uropathogens.
Study Design: Distributions of urinary isolates and their antibiogram
Place and Duration of Study: Mubi General Hospital, Adamawa State, between April, 2013 and January, 2014
Methodology: Urine samples of 101 patients comprised of 46 males and 55 females were analyzed for bacterial growth, antibiogram and multiple antibiotic resistance index.
Results: Females showed higher prevalence of UTI than males. Gram negative bacteria (61.7%) were found in high prevalence than Gram positive (29.3%). *Staphylococcus aureus* (58.3%) has the highest prevalence rate among Gram positive organisms, while *Citrobacter freundii* (25.3%) was the most prevalent Gram negative isolates. *Citrobacter freundii* (17.9%) was the most prevalent uropathogens closely followed by *S. aureus* (17.1%). Antimicrobial susceptibility was performed on all isolated bacteria by the disc diffusion method employing multiple antibiotic discs differently for both Gram positive and

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Gram negative isolates. The results showed that *S. aureus* and Coagulase Negative Staphylococci (CoNS) were more susceptible to Chloramphenicol (83%), followed by Streptomycin and Amoxicillin (78%). While their resistance profile showed that *S. aureus* and CoNS are more resistant to Ampiclox, Gentamycin and Rifampicin (31%). Susceptibility to all the antibiotics by Gram positive organisms was significantly higher than their resistance to the same antibiotics (p< 0.05). Gram negative organisms are more susceptible to Streptomycin (62%), followed by Ciprofloxacin (47%) and Ofloxacin (44%), while their resistance profile showed that they are more resistance to Nalixidic acid (79%) followed by Augmentin (76%), Ampicillin (75%) and Reflacine (74%). Resistance to all the antibiotics by Gram negative organisms is significantly higher than their susceptibility to the same antibiotics (p<0.05). Using spearman correlation, the results further showed significant correlation in resistance between *P. vulgaris*, *Escherichia coli* and *P. agglo*merans (p<0.01). Also, there was significant correlation in resistance between *E. coli*, *Klebsiella* sp., *Citrobacter diversus* and *P. vulgaris* (p<0.05). The multiple antibiotic resistances (MAR) index of each antibiotic was calculated. The MAR index for Gram positive antibiotics was significantly lower than that of Gram negative antibiotics (p<0.05).

Conclusion: In this study, we found multidrug resistance strains which are resistant to most of the antimicrobials agent tested more especially the Gram negative uropathogens. This reflected the fact that Nalixidic acid, Augmentin, Ampicillin, Reflacine, Ceporex and Septrin were the most commonly prescribed antibiotics in the hospital even before the results of urine analyses and also the most easily available in the market without prescription and because they were also very cheap in terms of cost. Consequently, the widespread use or misuse of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria.

Keywords: Urinary tract infection; Citrobacter freundii; antibiotic; susceptibility; resistance.

1. INTRODUCTION

Urinary tract infection (UTI) is an infection that affects part of the urinary tract. It is called cystitis (bladder infection) and pyelonephritis (kidney infection) when it affects the lower and upper urinary tracts respectively. Urinary tract infection is the most common bacterial infection with a high rate of morbidity and financial cost. It also includes the most common nosocomial infection in many hospitals and accounts for approximately 35% of all hospital acquired infections. In adults, persisting UTIs can lead to arterial hypertension and renal failure. Etiologic agents of UTIs are variable and usually depend on time, geographical location and age of patients. Although UTIs can be caused by any pathogenic organism from the urinary tract, the most frequent is family of Enterobacteriaceae, causing 84.3% of the UTIs [1,2]. UTI is more common in females than in males as female urethra structurally found less effective for preventing the bacterial entry [3]. It may be due to the proximity of the genital tract and urethra and short urethra of females [4] and adherence of urothelial mucosa to the mucopolysaccharide lining [5]. The other main factors which make females more prone to UTI are pregnancy and sexual activity [6]. In pregnancy, the physiological increase in plasma volume and decrease in urine concentration develop glycosuria in up to 70% women which ultimately leads to bacterial growth in urine [7]. Also in the non pregnant state the uterus is situated over the bladder whereas in the pregnant state the enlarged uterus affects the urinary tract [8]. Sexual activity in females also increases the risk of urethra contamination as the bacteria could be pushed into the urethra during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during child birth [9,10].

Worldwide data shows that there is an increasing resistance among uropathogens to conventional drugs. Resistance has emerged even to newer, more potent antimicrobial agents [11]. The epidemiology and the resistant patterns show a regional variability and prove to have a continuous change of frequency, due to excessive use of antibiotics. Studies show that the risk factors play an important role, for the emergence of the antibiotics resistance. Some of them are due to the mal-administration of the antibiotics in the past history, renal malformations associated and the frequent use of antibiotics for the prophylaxis of recurrent infections. However, many reports have indicated the presence of multidrug resistance in organisms causing UTIs.

2. MATERIALS AND METHOD

2.1 Study Population

The urine samples of 101 patients, comprised of 46 males and 55 females, who attended the outpatient departments (OPDs) of the Hospital and had clinical evidence of urinary tract infection, determined by urologists, were included in this study.

2.2 Sample Collection

Midstream urine was collected into a 20ml calibrated sterile screw-capped universal container which was distributed to the patients. The specimens were labeled appropriately, transported to the laboratory, and kept at 4^{o}c for further analyses. Verbal informed consent was obtained from all patients prior to specimen collection.

2.3 Sample Processing

A loopful urine sample was plated on MacConkey agar and replicated on Mannitol salt agar (Hi Media Laboratories, Mumbai, India) for differential purpose. The inoculated plates were incubated at 37°C for 24h and for 48h

2.4 Identification of Bacterial Isolates

Identification of bacterial isolates was done on the basis of their cultural and biochemical characteristics. Gram positive microorganisms were identified based on their reactions to coagulase and Catalase test and Mannitol test for *Staphylococcus aureus.* Gram negative bacteria were identified by standard biochemical tests [12]. The isolates were sub-cultured on nutrient agar slants periodically to maintain the pure culture.

2.5 Antibiotic Susceptibility Testing

Isolates were tested for antimicrobial susceptibility testing by the standard disc diffusion method. Standard inoculums adjusted to 0.5 McFarland was swabbed on Nutrient agar (Hi Media Laboratories, Mumbai, India) and was allowed to soak for 2 to 5 minutes. After that antibiotic discs were placed on the surface of media and pressed gently. Nutrient agar plates were then incubated at 37°C for 24h. Zones of inhibition were measured and interpreted by the recommendations of clinical and laboratory standards [13]. The following standard antibiotic discs were used for the isolates; ampiclox, gentamycin, chloramphenicol, norfloxacin, streptomycin, amoxicillin, rifampicin, levofloxacin, erythromycin and ciprofloxacin for Gram positive isolates. While streptomycin, septrin, ciprofloxacin, augmentin, gentamycin, reflacine, tarvid, ceporex, ampicillin and nalixidic acid for Gram negative bacterial isolates.

2.6 Multiple Antibiotic Resistance (MAR) Indexing

The multiple antibiotic resistance indices (MARI) were calculated by the method described by Tambekar et al. [14]. The following formula was used for the calculation of MAR index of antibiotics: MAR index for an antibiotic = (number of antibiotics resistant to the isolates/ (number of antibiotics × number of isolates). The number of MAR index for an antibiotic indicates its sensitivity and resistance. Antibiotic resistance increases with the increasing MAR values.

2.7 Statistical Analysis

Anova and Student T-test was used to test for significance difference in all the data obtained. All statistical analyses were carried out using the SPSS 17.0 window based program. Significance difference and Non-significance difference was defined when $p \leq 0.05$ and $p \geq 0.05$ respectively.

3. RESULT

In this study, 101 urine samples were analysed, out of which 123 organisms belonging to 13 genera were isolated. Forty (32.5%) of the isolates were from males, while 83 (67.5%) were from females. This result showed that the number of organisms isolated from females were significantly higher than those from male counterparts ($p<0.05$). In males, *Citrobacter freundii* followed by *S. aureus* and CoNS had highest prevalent rates, while in females *S. aureus* and *P. vulgari*s occur at the same rate and had the highest prevalent rate. The results revealed that 36 of the isolates are Gram positive while 87 are Gram negative. Among the Gram positive organisms, only *Staphylococcus aureus* and CoNS were isolated, out of which 14(38.9%) were isolated from males, while 22(61.1%) were isolated from females with *S. aureus* having the highest incidence rate. Ten (10) genera of Gram negative organisms comprising of 87 isolates were obtained; out of which 26(29.9%) were isolated from males while 61(70.1%) from females with *Citrobacter freundii* having the highest incidence rate followed by *Proteus vulgaris*. 80(91.9%) of the Gram negative isolates belong to the Enterobacteriaceae family.

C*. freundii* was found the dominant bacteria among all isolated uropathogens with the prevalence rate of 17.9%. The second most prevalent isolate was *S. aureus* (17.1%), followed by *Proteus vulgaris* (13.8), *E. coli* (13.0), Coagulase-negative Staphylococci (12.2%); while *Klebsiella* sp., *Shigella* sp. and *Serratia marcescens* showed the least incidence rate Table 1.

The results from Table 2 showed the antibiotic susceptibility profile of *S. aureus* and CoNS. *S. aureus* and CoNS were more susceptible to Chloramphenicol (83%), followed by Streptomycin and Amoxicillin (78%). while their resistance profile showed that *S. aureus* and CoNS are more resistant to Ampiclox, Gentamycin and Rifampicin (31%). The results of the statistical analyses revealed that *S. aureus* and CoNS are more susceptible to all the antibiotics when compared to their resistance to the same antibiotics ($p<0.01$). However, *S. aureus* showed higher susceptibility to all the antibiotics than CoNS ($p<0.01$). Contrary wise, resistance to all the antibiotics by CoNS was significantly higher than that of *S. aureus* ($p<0.01$).

Table 1. Prevalence of uropathogens according to gender

S/N	Organisms	Males	Females	Total (%)
1	*Staphylococcus aureus*	7	14	21(17.1)
2	CoNS	7	8	15(12.2)
3	*Escherichia coli*	4	12	16(13.0)
4	*Klebsiella* spp	1	2	3(2.4)
5	*Citrobacter freundii*	9	13	22(17.9)
6	*Citrobacter kosei*	6	2	8(6.5)
7	*Proteus vulgaris*	3	14	17(13.8)
8	*Proteus mirabilis*	1	3	4(3.3)
9	*Pseudomonas aeruginosa*	1	6	7(5.7)
10	*Shigella* spp	1	2	3(2.4)
11	*Pantoea agglomerans*	0	4	4(3.3)
12	*Serratia marcescens*	0	3	3(2.4)
	Total	40	83	123(100)

KEY: CoNS= Coagulase-negative staphyloccoci

Table 2. Antibiotic susceptibility pattern of *S. aureus* and Coagulase-negative staphylococci

S/N		*S. aureus*		CoNS		Total		MARI
	Antibiotics	S	R	S	R	S	R	
1	Ampiclox	16(76)	5(24)	9(20)	6(40)	25(69)	11(31)	0.031
2	Gentamycin	17(81)	4(19)	8(53)	7(47)	25(69)	11(31)	0.031
3	Chloramphenicol	19(90)	2(10)	11(73)	4(27)	30(83)	6(17)	0.017
4	Norfloxacin	17(81)	4(19)	10(67)	5(33)	27(75	9(25)	0.025
5	Streptomycin	18(86)	3(14)	10(67)	5(33)	28(78)	8(22)	0.022
6	Amoxicillin	18(86)	3(14)	10(67)	5(33)	28(78)	8(22)	0.022
7	Rifampicin	17(81)	4(19)	8(53)	7(47)	25(69)	11(31)	0.031
8	Levofloxacin	17(81)	4(19)	10(67)	5(33)	27(75)	9(25)	0.025
9	Erythromycin	18(86)	3(14)	9(60)	6(40)	27(75)	9(25)	0.025
10	Ciprofloxacin	18(86)	3(14)	9(60)	6(40)	27(75)	9(25)	0.025

MARI= Multiple Antibiotic Resistance Index

The antibiotic susceptibility profile of Gram negative organisms showed variation in their susceptibility and resistance to same antibiotics as shown in Table 3. Gram negative organisms are more susceptible to Streptomycin (62%), followed by Ciprofloxacin (47%) and Ofloxacin (44%); while their resistance profile showed that they were more resistance to Nalixidic acid (79%) followed by Augmentin (76%), Ampicillin (75%) and Reflacine (74%). *Shigella* sp. are 100% resistant to all the antibiotics except Streptomycin, Augmentin, and Nalixidic acid. *Salmonella* sp. are 100% resistant to Septrin, Augmentin, and Reflacine, while *P. agglomerans* are 100% resistant to Augmentin, Ampicillin, and Nalixidic acid. The results of the statistical analyses revealed that although resistance to Streptomycin by all Gram negative organisms was the least, but was not significantly different ($p>0.05$) from that of Ciprofloxacin, but significantly lower than that of the other antibiotics ($p<0.05$). Similarly, resistance to Ciprofloxacin is not significantly different from that of Gentamycin and Ofloxacin ($p>0.05$). Resistance to all the antibiotics by Gram negative organisms is significantly higher than their susceptibility to the same antibiotics ($p<0.05$). Using spearman correlation, the results further showed significant correlation in resistance between *P. vulgaris*, *Escherichia coli* and *P. agglo*merans ($p<0.01$). Also, there was significant correlation in resistance between *E. coli*, *Klebsiella* sp., *Citrobacter koseri* and *P. vulgaris* ($p<0.05$).

Table 3. Antibiotic susceptibility profile of Gram negative isolates

S/N	Isolates	Observations	Antibiotics									
			Steptomycin	**Septrin**	**Ciprofloxacin**	**Augmentin**	**Gentamycin**	**Reflacine**	**Ofloxacin**	**Ceporex**	**Ampicillin**	**Nalixidic acid**
1	*E. coli*	S	9(56)	7(44)	11(69)	6(37)	8(50)	6(37)	8(50)	3(19)	7(44)	2(12)
		R	7(44)	9(56)	5(31)	10(63)	8(50)	10(63)	8(50)	13(81)	9(56)	14(88)
2	*K. pneumoniae*	S	3(100)	2(67)	2(67)	2(67)	2(67)	2(67)	2(67)	0	1(33)	0
		R	0	1(33)	1(33)	1(33)	1(33)	1(33)	1(33)	3(100)	2(67)	3(100)
3	*C. freundii*	S	11(50)	3(14)	4(18)	6(27)	5(29)	2(9)	8(36)	6(27)	4(18)	4(18)
		R	11(50)	19(86)	18(82)	16(73)	17(71)	20(91)	14(64)	16(73)	18(82)	18(82)
4	*C. kosei*	S	5(63)	4(50)	5(63)	1(12)	2(25)	1(12)	3(37)	3(37)	3(37)	2(25)
		R	3(37)	4(50)	3(37)	7(88)	6(75)	7(88)	5(63)	5(63)	5(63)	6(75)
5	*P. vulgaris*	S	10(59)	5(29)	8(47)	1(6)	5(29)	4(24)	6(35)	3(18)	1(6)	4(24)
		R	7(41)	12(71)	9(53)	16(94)	12(71)	13(76)	11(65)	14(82)	16(94)	13(76)
6	*P. mirabilis*	S	4(100)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)
		R	0	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)
7	*P. aeruginosa*	S	5(71)	2(29)	4(57)	2(29)	4(57)	5(71)	5(71)	3(43)	3(43)	2(29)
		R	2(29)	5(71)	3(43)	5(71)	3(43)	2(29)	2(29)	4(57)	4(57)	5(71)
8	*Shigella* sp	S	1(33)	0	0	1(33)	0	0	0	1(33)	0	1(33)
		R	2(67)	3(100	3(100)	2(67)	3(100)	3(100)	3(100)	2967)	3(100)	2(67)
9	*P. Agglomerans*	S	3(75)	1(25)	3(75)	0	3(75)	1(25)	1(25)	1(25)	0	0
		R	1(25)	3(75)	1(25)	4(100)	1(25)	3(75)	3(75)	3(75)	4(100)	4(100)
10	*Salmonella* sp	S	3(100)	0	2(67)	0	2(67)	0	3(100)	2(67)	1(33)	1(33)
		R	0	3(100)	1(33)	3(100)	1(33)	3(100)	0	1(33)	2(67)	2(67)
11	TOTAL	S	54(62)	26(30)	41(47)	21(24)	33(38)	23(26)	38(44)	24(28)	22(25)	18(21)
		R	33(38)	61(70)	46(53)	66(76)	54(62)	64(74)	49(56)	63(72)	65(75)	69(79)
12	MAR INDEX		0.038	0.070	0.053	0.076	0.062	0.074	0.056	0.072	0.075	0.079

KEY: MAR INDEX= Multiple antibiotic resistance index

The Multiple antibiotic resistance (MAR) index for *S. aureus* and CoNS, was found for ampiclox, gentamycin and rifampicin (0.031 each) indicating that these antibiotics were highly resistant among all tested Gram positive isolates; however the lowest MAR index was found for Chloramphenicol (0.017) followed by Streptomycin and Amoxicillin (0.022) indicating the highest sensitivity against the tested isolates Table 2. Also, for Gram negative uropathogens, the highest MAR index was found for Nalixidic acid (0.079), followed by Augmentin (0.076) and Ampicillin (0.075) which indicates that these antibiotic were highly resistant among the Gram negative uropathogens tested; however, the lowest MAR index was found for Streptomycin (0.038) and Ciprofloxacin (0.053) indicating highest sensitivity against the tested isolates Table 3. Although both Gram positive and Gram negative organisms were tested with different antibiotics, the MAR index for Gram positive organisms was significantly lower than that of Gram negative organisms ($p<0.05$).

4. DISCUSSION

Our study showed a high prevalence of UTI in females (73.57%) than in males (35.14%) which correlate with other findings which revealed that the frequency of UTI is greater in females as compared to males [4,15-18]. The reason behind this high prevalence of UTI in females may be due to close proximity of the urethral meatus to the anus, shorter urethra, sexual intercourse, incontinence, and bad toilet [19-21].

In this study, the Gram negative bacilli constituted 70.7% of the total bacterial isolates while Gram positive cocci constituted 29.3%. This was in agreement with earlier report which states that Gram-positive cocci had a comparatively low contribution in causing UTIs [22]. Out of the 87 (70.3%) Gram negative uropathogens isolated in this study, Enterobacteriaceae isolates (91.9%) were the dominant bacterial species isolated from urine cultures which was in agreement with previous works [2,23,1,22]. Higher incidence of Gram negative bacteria, related to Enterobacteriaceae, in causing UTI has many factors which are responsible for their attachment to the uroepithelium. In addition, they are able to colonize in the urogenital mucosa with adhesins, pili, fimbriae, and P-1 blood group phenotype receptor [24].

Citrobacter freundii (25.3%) was found the most prevalent Gram negative bacteria in the positive urine samples of UTI. While *Citrobacter freundii* and *Citrobacter koseri* constitute 34.5% of total Gram negative bacterial isolates from this study. In accordance with this study, Pan et al. [25] from China and Mohanty et al. [26] from India reported high prevalence of *Citrobacter spp.* in different clinical samples of their study with urinary tract infections as the most common associated clinical syndromes. In Nigeria, there was no report of *Citrobacter* spp as the most prevalent uropathogens. Thus, the findings of this study serve as the baseline report of *Citrobacter freundii* as the most prevalent bacterial isolates from urinary tract infections. Contrary to the findings of this study however, most studies around the world showed that *E. coli* is the most prevalent uropathogens [27-32]. Other studies showed that *Klebsiella* spp. [33] and *Pseudomonas aeruginosa* [34] are the most prevalent uropathogens. Other studies showed that *Citrobacter* spp. was either the second most prevalent uropathogens [35,36] or the third most prevalent urinary isolates [37,38]. In the same vein, many other studies revealed the changing patterns in the etiological agents of urinary tract pathogens and their sensitivities to commonly prescribed antibiotics [39-41]. These changing pattern or variability usually depend on time, geographical location and age of patients [1]. Although *Citrobacter* spp. are less commonly isolated, they are emerging as a common nosocomial multidrug‑resistant pathogen, especially in developing countries. The predisposing factor is a weak and attenuated immune system and functioning of the body. A frail immune system makes the body more vulnerable and predisposed to *C. freundii*, thus triggering UTI. The isolation of this organism may also be associated with catheterization, genitourinary instrumentation, or obstructive uropathy [39]. Thus, the isolation of *Citrobacter freundii* as the most prevalent uropathogen in this study is justified and may be attributed to any of the factors outline above.

Citrobacter can cause a wide spectrum of infections in humans, such as infections in the urinary tract, respiratory tract, wounds, bone, peritoneum, endocardium, meninges and blood stream [42,43]. Among the various sites of infection, the urinary tract is the most common [44].

The findings that *S. aureus* is the second most predominant uropathogens as shown in this study was supported by previous studies [45,46].

Moreover, the findings that *Klebsiella* spp., *Staphylococcus aureus,* and *Salmonella* spp. are found rarely in UTIs as reported by Foxman and Brown, [47] was consistent with the present study with regard to *Klebsiella* spp. and *Salmonella* spp, but went contrary to the findings of this study with regard to *Staphylococcus aureus*. In the present study, *Staphylococcus aureus* is the second most prevalent UTI pathogen, while *Klebsiella* spp. was the least; *Salmonella* spp. was not even isolated in our study.

Worldwide data shows that there is an increasing resistance among UTI pathogens to conventional drugs. Resistance has emerged even to newer, more potent antimicrobial agents [11]. The antibiotic susceptibility profile of *S. aureus* and CoNS showed high sensitivity to all the tested antibiotics which ranged from 69%-83% with Chloramphenicol being the most effective antibiotics. Contrary to this study however, Khan and Zaman, [48] showed that 60-79% of urinary isolates in their study were resistant to Chloramphenicol. In this study, *S. aureus* and CoNS were more resistance to Ampiclox, Gentamycin and Rifampicin (31%) with MAR index of 0.031 each. The findings of this study slightly went contrary to the report of Manikandan et al. [46] which revealed that *S. aureus* from urinary source showed sensitivity to Gentamycin in addition to other antibiotics. In this study also, 25% *S. aureus* and CoNS were resistance to Ciprofloxacin, Erythromycin, Levofloxacin and Norfloxacin. Contrary to this study, Khan and Zaman [48] showed that 60-79% of their urinary isolates were resistant to the same antibiotics.

The antibiotic resistance profile of Gram negative uropathogens revealed that the isolates are mostly resistance to Nalixidic acid (79%) followed by Augmentin (76%), Ampicillin (75%), Reflacine (74%), Ceporex (72%) and Septrin (70%). This finding is similar to the report of Manikandan et al. [46] that showed 83.3% and 80.6% of their urinary isolates were resistance to Septrin and Nalixidic acid respectively. It has been reported by Sahm et al. [49] that Ampicillin has no more effect on any of the isolates of UTI. Our study also revealed that 75% of isolates are resistance to Ampicillin, which may be due to the frequent-haphazard use of Ampicillin. In agreement with the finding of this study, Khan and Zaman, [48] showed that 90% of their urinary isolates were resistant to Ampicillin. Resistance to Aminoglycosides (streptomycin 38%, and gentamycin 62%) in our study was relatively high with respect to Gentamycin, which was contrary to some reports on UTIs [1,22,46]. Although in this study, resistance to Streptomycin was the least with MAR index of 0.038; this showed that Streptomycin was the most effective drug in our study against Gram negative uropathogens and can be used to manage UTIs involving these organisms in our study area. This finding was also supported by previous studies [48,50] which showed that Streptomycin might be the drug of choice to treat UTI.

4. CONCLUSION

In this study, we found multidrug resistance isolates which are resistant to more than one antibiotic more especially the Gram negative uropathogens. This reflected the fact that Nalixidic acid, Augmentin, Ampicillin, Reflacine, Ceporex and Septrin were the most commonly prescribed antibiotics in the hospital even before the results of urine analyses and also the most easily available in the market without prescription and because they were also very cheap in terms of cost. Consequently, the widespread use or misuse of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria.

CONSENT

Verbal informed consent was obtained from all patients prior to specimen collection.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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