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Original Research Article

Sensitivity and resistance patterns of gram-negative uropathogens isolated from the urine of patients with upper/lower urinary obstruction in Nigeria.

Abstract

Background: Urinary tract infection is a cause of significant morbidity and potential mortality in patients. Urine microscopy culture and sensitivity enable the isolation of the incriminating microbes. The sensitivity and resistance of the various microorganisms are invaluable in the effective management of UTIs and the associated adverse consequences. Gram-negative organisms are the usual pathogens responsible for most UTIs. The abuse of antibiotics can increase the prevalence of antimicrobial resistance. This leads to an increased cost of treatment, as more expensive higher-end antibiotics may become indicated. There is also the risk of spreading multidrug-resistant infections to the community.

Aims: To evaluate the sensitivity and resistance patterns of commonly available antibiotics to uropathogens in the positive urine culture of patients who presented with upper and lower urinary obstruction.

Methods: This retrospective study was carried out on urine samples of patients with upper/lower urinary obstructive from two specialist urology referral hospitals with a positive culture and sensitivity tests between January 2011 and December 2020. The patients' case notes were retrieved, and their urine culture, sensitivity results and mode of treatment were analyzed. These data were collated using Microsoft Excel, and they were analysed using SPSS version 20.

Results: 314 urine samples had positive culture and sensitivity tests. All were Gram-negatives: *Klebsiella, Escherichia Coli, Pseudomonas, Proteus* and *Citrobacter spp*. in decreasing frequency. Among the quinolones, levofloxacin {56.7% (178)} had the highest moderate-high

(M-H) sensitivity to the Gram-negative uropathogens; followed by Ciprofloxacin {46.2 % (145)} and Ofloxacin {19.1% (60)}.

The gram-negatives were most sensitive to streptomycin {75.5% (237)} and gentamicin 62.4% (196)}and also least resistant to them. (Streptomycin 11.1%; gentamicin 21.0%) The highest resistance was to Nalidixic acid {90.1%, (225)}, peflacine {76.1% (239)}, Augmentin {73.6% (231)} and Ampicillin{72%(226)}.

Conclusion: Among the commonly available antibiotics in our study, the gram-negative uropathogens are the most sensitive and least resistant to streptomycin, gentamycin and levofloxacin. Levofloxacin had the best sensitivity and lowest quinolones resistance compared to ciprofloxacin and ofloxacin. There is very low sensitivity and high resistance to nalidixic acid, ampicillin, Augmentin, Septrin and Peflacine.

Keywords: Bacteria, gram-negative, sensitivity, resistance, UTI

Introduction

Urinary Tract Infection (UTI) is the inflammatory response of the urothelium to microbial invasion.[1] UTIs are quite common and affect men, women, young, old, immunocompetent and immunocompromised. The urinary tract should usually be free of microorganisms. Bacteria can ascend from the perineum and lead to inoculation, adherence, colonization and infection.[2] These processes are more likely to occur when host defence mechanisms are reduced or the virulence of the organisms increases. UTIs can also happen following haematogenous spread.[3]

The infection may be asymptomatic or symptomatic. In symptomatic individuals, it can cause storage symptoms, painful voiding and severe life-threatening pyelonephritis associated with pyrexia, nausea, vomiting, and rigours. Renal abscess, perinephric abscess and urosepsis can also occur following UTI. These can lead to significant morbidity, may progress to renal scarring and end-stage renal failure.[4]

The common organisms that cause UTIs include Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, Citrobacter spp and Staphylococcus saprophyticus. Effective treatment requires evaluation with a careful history, examination, urine culture and sensitivity, and identifying the risk factor for urinary obstruction.[5.] This ensures that an appropriate antibiotic is utilized to treat the cultured bacteria and prevent the development of resistant strains.[6]

Antibacterial resistance is known to increase morbidity, mortality, and cost of treatment.[7,8,9] As observed in our environment, indiscriminate use and abuse of antibiotics can lead to an increased prevalence of antimicrobial resistance. This increases the cost of treatment, as more expensive higher-end antibiotics may become indicated. There is also the risk of spreading multidrug-resistant infections to the community.[9] We aim to evaluate the sensitivity and resistance patterns to the commonly available antibiotic by uropathogenic bacteria in the urine culture of patients who presented with urine stasis.

Materials and Methods

This retrospective study was carried out on urine samples of patients with upper/lower urinary obstructive from two specialist urology referral hospitals with a positive culture and sensitivity tests between January 2011 and December 2020. The patients presented to the Urology

Department University of Port Harcourt Teaching Hospital, and Rosivylle Clinic and Urology Centre, Port Harcourt, River, Nigeria. All who had a positive culture with clinical or imaging features of upper and lower urinary tract obstruction and stasis were included in the study. The folders were retrieved, and their age, sex, urine culture and sensitivity results, and mode of treatment were analyzed. The culture and sensitivity tests were carried out using gram-negative and gram-positive culture discs. The degree of sensitivity is quantified as +1= low sensitivity; +2= moderate sensitivity; +3= high sensitivity; Mild to Moderate sensitivity = M-M and Moderate to High sensitivity = M-H. Patients with incomplete records and without sensitivity reports were excluded from the study. These data were collated using Microsoft Excel version 2016, and they were analyzed using SPSS version 20.

Results

Three hundred and fourteen patients had uropathogens cultured from their urine samples. The organisms were all gram-negative: *Escherichia coli, Klebsiella sp., E. Coli, Pseudomonas spp, Proteus spp* and *Citrobacter spp*. in decreasing order of frequency. The gram-negative uropathogens had the highest activity and lowest resistance to streptomycin, gentamicin and rifampicin. The lowest sensitivity and highest resistance were observed with nalidixic acid and the Penicillin -Ampicillin and Augmentin.

The antibiotic with the highest sensitivity was streptomycin, with the cultured organisms expressing M-H in 75.5% (237) and resistance of 11.1%(35). M-H sensitivity to gentamicin was noted in 62.4% (196), and resistance was observed in 21.0% (66).

Levofloxacin had the best activity on the gram-negative organisms of the quinolones, with 56.7% (178) M-H sensitivity and 20.4% (64) resistance. Ciprofloxacin had M-H sensitivity to the uropathogens and was observed in only 46.2% (145), with resistance seen in 27.7% (87)

10.9% (34) of the gram-negative organism had M-H sensitivity to ampicillin; 25.5% (80) had M-H sensitivity to Amoxicillin, and 9.6% (30) were M-H sensitive to Augmentin.

Nalidixic acid had the least sensitivity, and the uropathogens all showed the highest resistance against it. The M-H sensitivity to nalidixic acid was only 4.1% (13) of the cultured uropathogens. 90.1% (283) of the gram-negative organism were resistant to nalidixic acid.

Table 1. Combined sensitivity and resistance pattern of uropathogens to common antibiotics.

Sensitivity and resistance pattern to various antibiotics. *(+1= Mild sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

SENSITIVITY/RESISTANCE	1+	2+	3+	Resistance
CIPROFLOXACIN	82(26.1)	95 (30.3)	50 (15.9)	87 (27.7)
NORFLOXACIN	52(16.6)	43 (13.7)	12 (3.8)	207 (65.9)
GENTAMICIN	52(16.6)	153 (48.7)	43 (13.7)	66 (21.0)
AMOXICILLIN	53(16.9)	59 (18.8)	21 (6.7)	181 (57.6)

STREPTOMYCIN	42(13.4)	145 (46.2)	92 (29.3)	35 (11.1)
PEFLACINE	70(22.3)	52 (16.6)	13 (4.1)	179 (57.0)
RIFAMPICIN	96(30.6)	58 (18.5)	82 (26.1)	78 (24.8)
ERYTHROMYCIN	95(30.3)	70 (22.3)	25 (8.0)	124 (39.5)
CHLORAMPHENICOL	64(20.4)	102 (32.5)	49 (15.6)	99 (31.5)
AMPICLOX	72(22.9)	51 (16.2)	11 (3.5)	180 (57.3)
LEVOFLOXACIN	72(22.9)	120 (38.2)	58 (18.5)	64 (20.4)
TARIVID	10(33.4)	47 (15.0)	13 (4.1)	149 (47.5)
REFLACINE	57(18.2)	15 (4.8)	3 (1.0)	239 (76.1)
AUGMENTIN	53(16.9)	25 (8.0)	5 (1.6)	231 (73.6)
NALIDIXIC ACID	18(5.7)	11 (3.5)	2 (.6)	283 (90.1)
SEPTRIN	51(16.2)	34 (10.8)	4 (1.3)	225 (71.7)
AMPICILLIN	54(17.2)	31 (9.9)	3 (1.0)	226 (72.0)

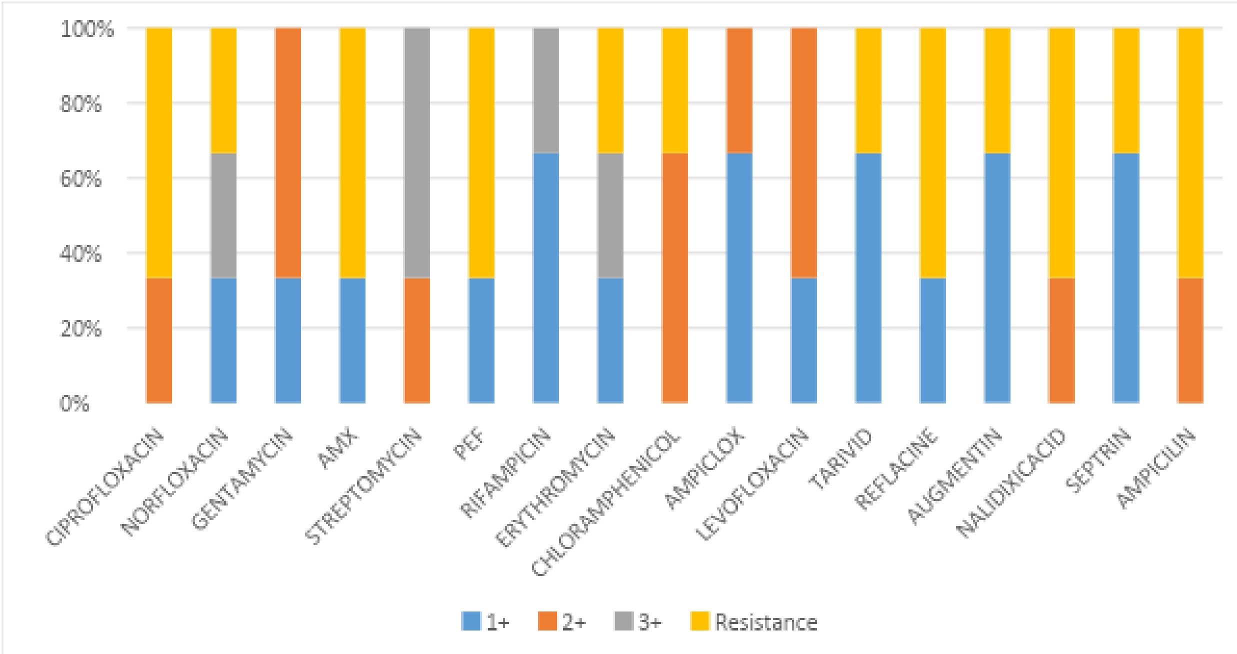

Figure 1. Sensitivity and resistance pattern of *Citrobacter spp.* to antibiotics. *(+1= Mild sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

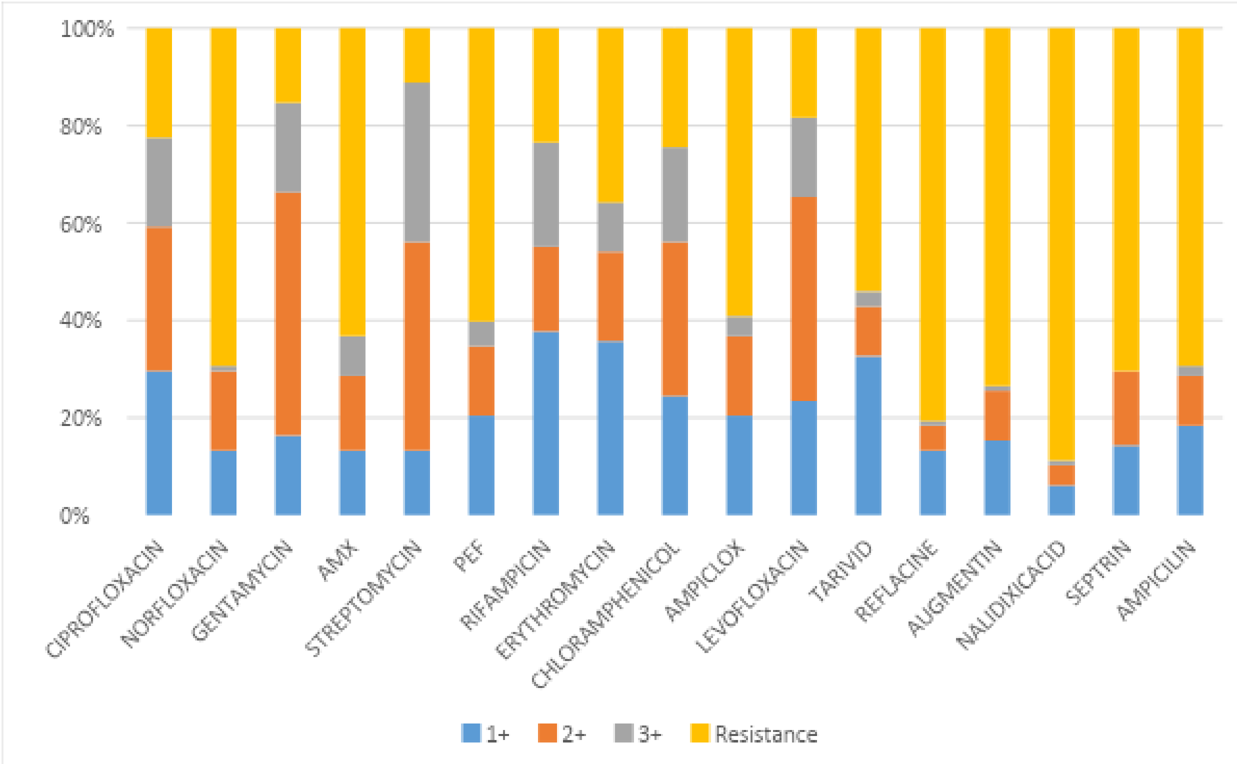

Figure 2. Sensitivity and resistance pattern of *Escherichia Coli* to common antibiotics. *(+1= Low sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

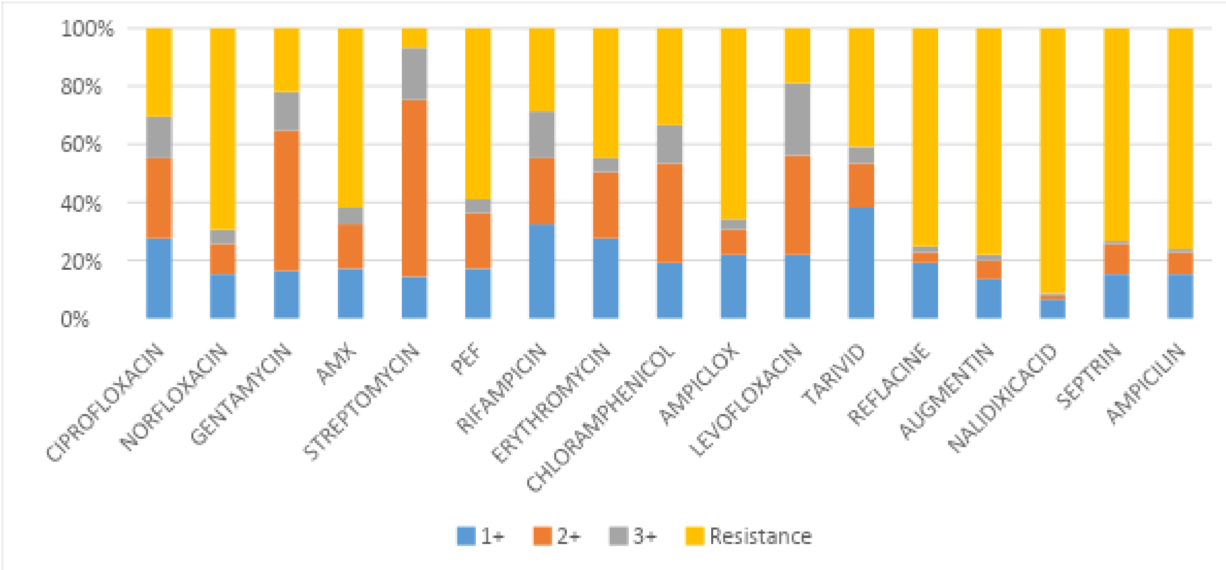

Figure 3. Sensitivity and resistance pattern of *Klebsiella sp*. to common antibiotics. *(+1= Low sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

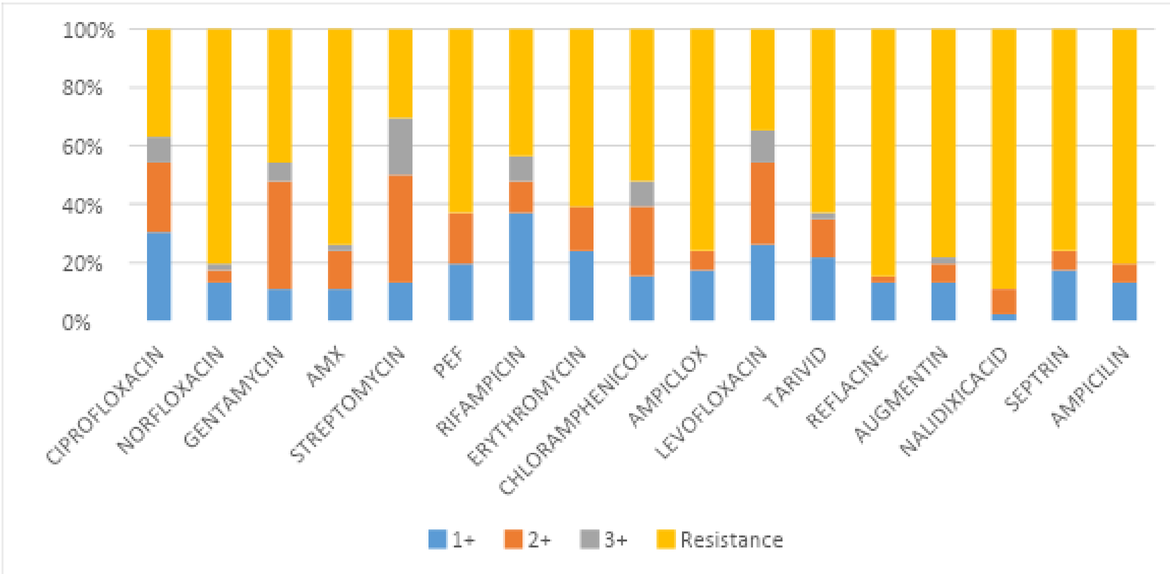

Figure 4. Sensitivity and resistance pattern of *Pseudomonas sp.* to common antibiotics. *(+1= Low sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

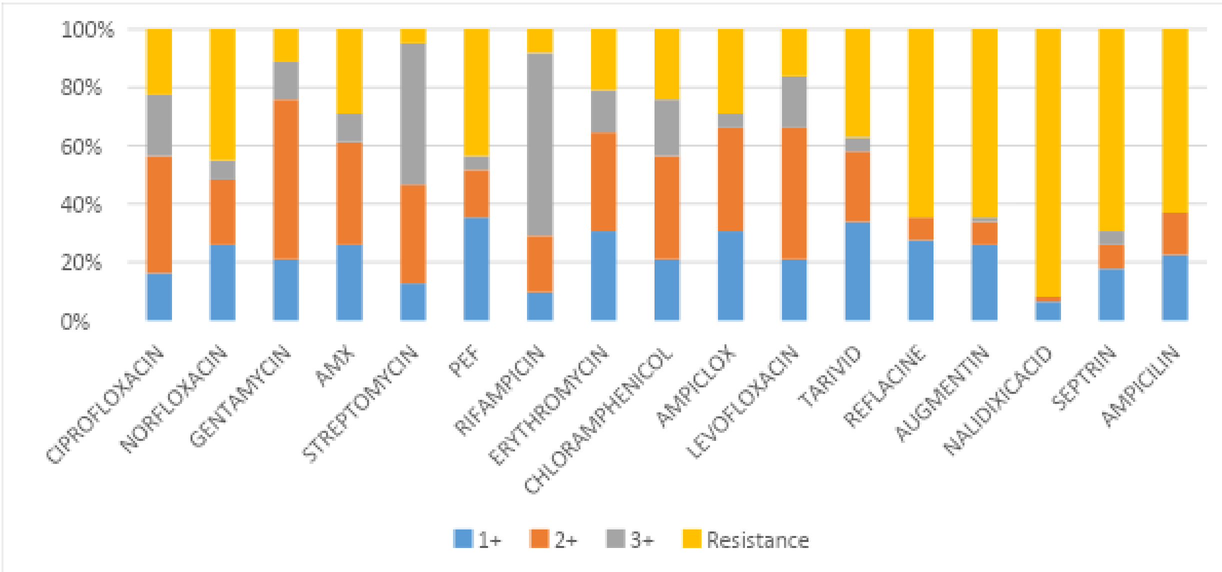

Figure 5. Sensitivity and resistance pattern of *Proteus sp.* to antibiotics. *(+1= Low sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

Discussion

The treatment objective of UTIs is essentially to eliminate proliferating bacteria in the urinary tract, which usually occurs within hours of administering the appropriate antibiotic. This underscores the invaluable premium and critical importance of using the right antibiotics during antimicrobial therapy. It should be excreted in the urine for the antibiotic to be effective. The level should be above the minimum inhibitory concentration (MIC) for the infecting organism.[10]

The activity of antimicrobial agents, besides the side effect profiles, is the most crucial consideration in managing UTIs.

Gram-negative organisms are the commonest organisms cultured in the urine from most studies worldwide in both sexes.[11-17] The route of infection is ascending from the perineum, from its situation near the anus. Uropathogens use different mechanisms for survival once in the urinary tract in response to stresses in the bladder, such as starvation and immune responses. By forming biofilms and undergoing morphological changes, uropathogens can persist and cause recurrent infection.[18,19]

Streptomycin is the first discovered aminoglycoside antibiotic, originally isolated from the bacteria *Streptomyces griseus*.[20] It is now used mainly in the treatment of tuberculosis. It has additional activity against gram-negative organisms hence its sensitivity to uropathogens.[21] The primary mechanism of action is inhibition of protein synthesis.[22] In this study, streptomycin was found to have the highest sensitivity and least resistance to the uropathogenic gram-negative organisms. (Tables 1 and Figures 1-5) The drug is administered via the parenteral route, and abuse is seldom. It is also ototoxic and nephrotoxic and should be used with caution, especially with other aminoglycosides. It is essential in tuberculosis treatment, and hence routine use for the treatment of UTIs may not be advisable. Such use can lead to resistance to uropathogens and increase the prevalence of multidrug drug-resistant tuberculosis. A common mechanism of bacterial resistance is via downregulation of drug uptake and modification of enzymes expressed by the bacteria.[23] A possible reason for the high sensitivity and low resistance of streptomycin among the gram-negative organisms is the restrictive or near-exclusive use for tuberculosis treatment. Also, abuse is expected to be less since it is a parenteral medication and is less utilized than readily available oral medications.

In our study, gentamicin had the second-best activity on the uropathogens, with an M-H sensitivity of 75.5% (237) and a low resistance of 13.7% (43). It is also used parenterally only, and hence it is less likely to be abused. Its mechanism of action, side effects, and development of resistance are similar to rifampicin, the second most sensitive antibiotic in this study.

Rifampicin was discovered in 1965 by Professor Piero Sensi.[24] It is on the World Health Organization's list of essential medicines. It is made by the soil bacterium *Amycolatopsis rifamycinia*.[25] The primary mechanism of action of gentamycin and rifampicin is the inhibition of bacterial DNA-dependant RNA polymerase.[24] The drug is used mainly in treating tuberculosis but can also treat leprosy, legionnaires and uropathogens in urine.[26] Rifampicin can cause hepatotoxicity leading to elevation of liver enzymes. It turns urine, sweat and tears red or orange. Rifampicin is intrinsically resistant to Enterobacteriaceae and pseudomonas specie,[27.] However, we found the activity of rifampicin against the uropathogens and resistance of 24.8% (78) to be relatively better than many of the other antibiotics in our study, likely due to its restricted use.

Levofloxacin is a broad-spectrum antibiotic that belongs to the drug class fluoroquinolone.[28] It is a left-handed isomer of the medication ofloxacin.[29] It is used to treat many bacterial infections, including UTIs. Its primary mechanism of action is the inhibition of DNA gyrase.[23] The main side effects include dizziness, gastrointestinal symptoms, myalgia and tendon rupture.[28] It is not routinely indicated in children because of premature fusion of the growth plate and cartilage problems. Levofloxacin is the third most sensitive antibiotic in this study, with an M-H sensitivity of 56.7% (178). Resistance was noted in 20.4% (64) and was the lowest among our study's oral antibiotics. Its mechanism of developing resistance is via active efflux of the drug, mutation in DNA gyrase binding site and alteration of cell wall porins.

Ofloxacin, pefloxacin and Norfloxacin (other fluoroquinolones) were found not to be as sensitive as levofloxacin and with the gram-negative organism showing high resistance as indicated in *Table* 1, *Figures 1-5.*

Nalidixic acid is also a synthetic quinolone and had the least sensitivity to the gram-negative microbes, with an M-H of only 4.1%, with 90.1% of the organisms resistant in our study. It is frequently used as a urinary antiseptic. Circumspection should be exercised with its use, given the observed present level of resistance in our environment.

Ampicillin is a Beta-lactam antibiotic used to manage and treat certain bacterial infections. It is in the aminopenicillin class of medications. Its mechanism of action is via inhibition of cell wall synthesis, and it causes cell wall lysis and death.[30] It can be administered through the oral, intramuscular and intravenous routes. Resistance is through the production of β-lactamase, changes in cell wall porin size and alteration of the penicillin-binding protein.[23] In this study, ampicillin was the second least sensitive antibiotic. Several other studies have noted antimicrobial resistance to ampicillin.[31,32] In our environment, ampicillin is readily bought over the counter, and it is taken orally in most cases. These may account for the low activity and high resistance rate of uropathogenic bacteria.

Besides the biological activity of the antibiotics, it appears from our study that oral antibiotics that are frequently used in the treatment of upper respiratory tract infections, such as the penicillin, augmentin, ampiclox, and pefloxacin, display low activity and high resistance to gram-negative organisms compared to the less frequent utilized medication like streptomycin, rifampicin, and gentamycin that are given parenterally. This emphasizes the importance of enforcing and strengthening the relevant regulatory bodies to help curtail the indiscriminate use and abuse of antibiotics to combat antibiotic resistance.

Conclusion

Among the commonly available antibiotics in our study, the gram-negative uropathogens are most sensitive and least resistant to streptomycin, gentamycin and levofloxacin. Levofloxacin had the best sensitivity and lowest quinolones resistance compared to ciprofloxacin and ofloxacin. There is very low sensitivity and high resistance to nalidixic acid, ampicillin, augmentin, septrin and peflacine. Active joint institutional and governmental effort is needed to combat the abuse of antibiotics that fosters resistance.

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