`

Sensitivity and resistance pattern of gram-negative uropathogens cultured from the urine of patients with upper and lower urinary obstruction.

Abstract

Background: Urinary tract infection is a cause of significant morbidity and potential mortality in some patients. Urine microscopy culture and sensitivity enable the isolation of the incriminating microbe. The sensitivity and resistance of the various microorganism are invaluable in the effective management of UTIs and the associated adverse consequences. Gram-negative organisms are the usual organism responsible for most UTIs. 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 urine culture of patients who presented with upper and lower urinary obstruction.

Methods and Methodology: This retrospective study was carried out on urine samples of patients from two specialist urology referral hospitals who had culture and sensitivity testing associated with urine stasis between January 2011 and December 2020. Patients with available records over the study period presented to the Urology department University of Port Harcourt Teaching Hospital and a Rosivylle Clinic and Urology Centre, Port Harcourt, Rivers State, with features of urinary tract infection associated with upper and lower urinary tract obstruction and stasis were included in the study. The patients' case notes were retrieved, and their age, sex, urine culture and sensitivity results, and mode of treatment were analyzed. Patients with

incomplete records were excluded from the study. These data were collated using Microsoft Excel, and they were analysed using SPSS version 20.

Results: There were three hundred and fourteen urine samples that had culture and sensitivity testing and had a positive growth of gram-negative uropathogens. They were *Klebsiella, E. Coli, Pseudomonas, Proteus* and *Citrobacter spp*. in decreasing order of 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)}. Levofloxacin had the best activity and least resistance {20.4% (64)}, followed by ciprofloxacin {27.7% (87)}and ofloxacin {47.5% (149). The gram-negative uropathogens were most sensitive to the parenteral aminoglycosides- streptomycin {75.5% (237)} and gentamycin 62.4% (196)}; they also had the least resistance among all the antibiotics. (Streptomycin 11.1%; gentamycin 21.0%) The highest resistance was to nalidixic acid {90.1%, (225)}, peflacine {76.1% (239)}, augmentin {73.6%(231)} and ampicillin{72%(226)}. E. Coli, Klebsiella and pseudomonas were all generally most sensitive to streptomycin, gentamycin and levofloxacin and mostly resistant to nalidixic acid peflacine and the penicillins. (ampicillin, Amoxycillin and augmentin)

Conclusion: Among the commonly available antibiotics, our study indicates that levofloxacin has the best sensitivity and lowest resistance among the quinolones compared to ciprofloxacin and ofloxacin. The gram-negative uropathogens are most sensitive and least resistant to streptomycin, gentamycin and levofloxacin. They had the lowest 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 defense 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 from patients who presented with urine stasis between January 2011 and December 2020. The patients with available records over the study period presented to the Urology Department University of Port Harcourt Teaching Hospital, and Rosivylle Clinic and Urology Centre, Port Harcourt, River, with urinary tract infection features associated with 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 analysed. 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 were excluded from the study. These data were collated using Microsoft Excel version 2016, and they were analysed using SPSS version 20.

Results

Three hundred and fourteen patients had uropathgen cultured from their urine samples. The organisms were all gram-negative: *Escherichia coli KlebFigures, E. Coli, Pseudomonas, Proteus* and *Citrobacter spp*. in decreasing order of frequency. Streptomycin, gentamycin and rifampicin had the highest sensitivity and lowest resistance to the gram-negative uropathogens. The lowest sensitivity and highest resistance were observed with nalidixic acid and the penicillins

(Ampicillin, Augmentin). Levofloxacin was the oral antibiotic with the highest activity with the gram-negative organisms.

Penicillin

10.9% (34) of the gram-negative organism had moderate to high sensitivity to ampicillin; 25.5% (80) had moderate to high sensitivity to Amoxicillin, and 9.6% (30) were moderate to highly susceptible to Augmentin. Resistance to the penicillins was high and noted in 72.0%, 57.6%, and 73.6% for ampicillin, Amoxil, and Augmentin, respectively.

Aminoglycosides

The antibiotic with the highest sensitivity was streptomycin, with the cultured organisms expressing moderate to high sensitivity in 75.5% (237). It also had the least resistance of all the antibiotics in our study, noted in 35 (11.1%) isolates. Moderate to high sensitivity to gentamycin was noted in 62.4% (196), and resistance was observed in 21.0% (66).

Quinolones

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

Nalidixic acid had the least sensitivity, and the uropathogens all showed the highest resistance against it. Moderate to high (M-H) sensitivity to nalidixic acid was noted in 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)
GENTAMYCIN	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)

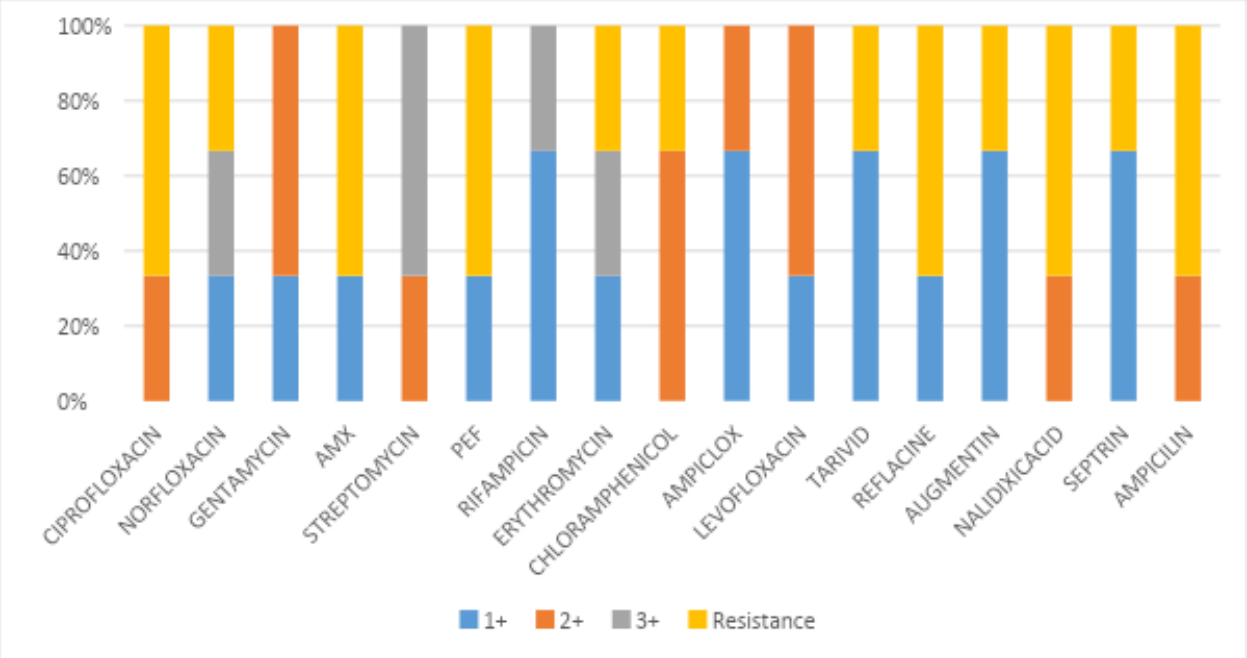

Figure 1. Sensitivity and resistance pattern of *Citrobacter spp.* to antibiotics. *(+1= Mild sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

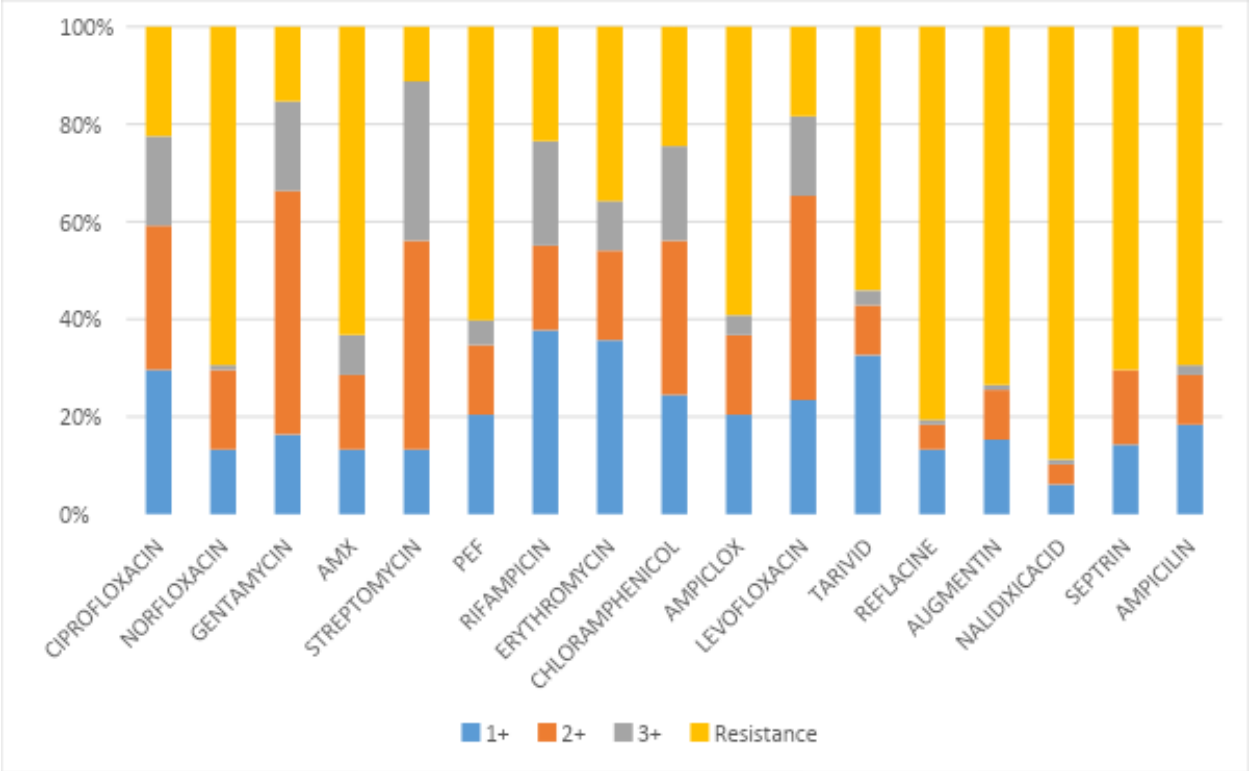

Figure 2. Sensitivity and resistance pattern of *Escherichia Coli* to common antibiotics. *(+1= Low sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

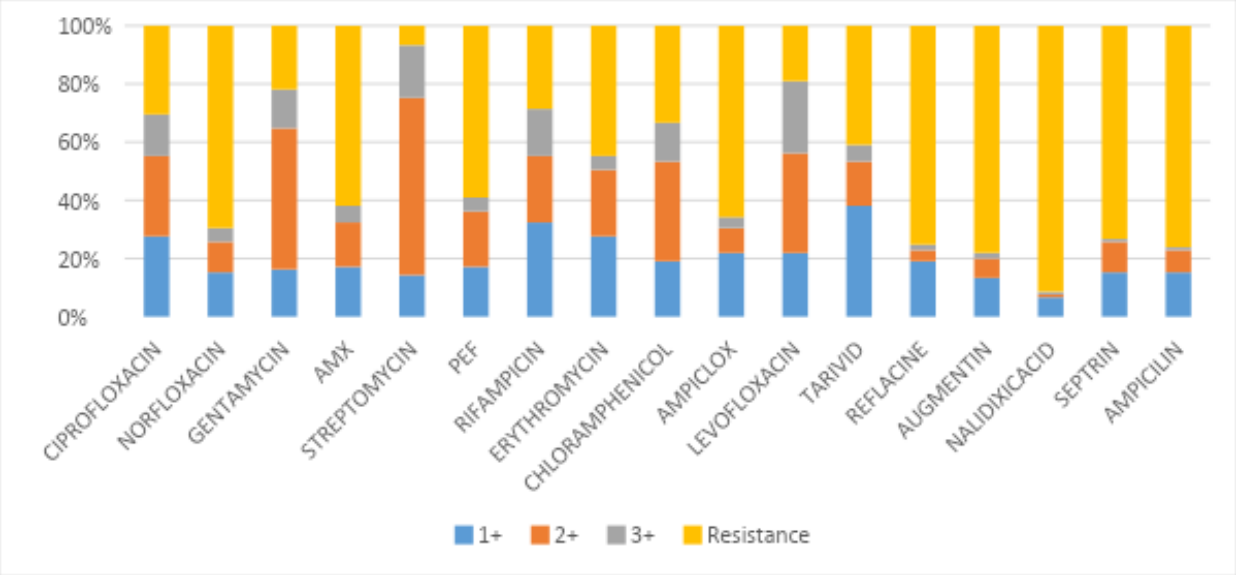

Figure 3. Sensitivity and resistance pattern of *Klebsiella sp*. to common antibiotics. *(+1= Low sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

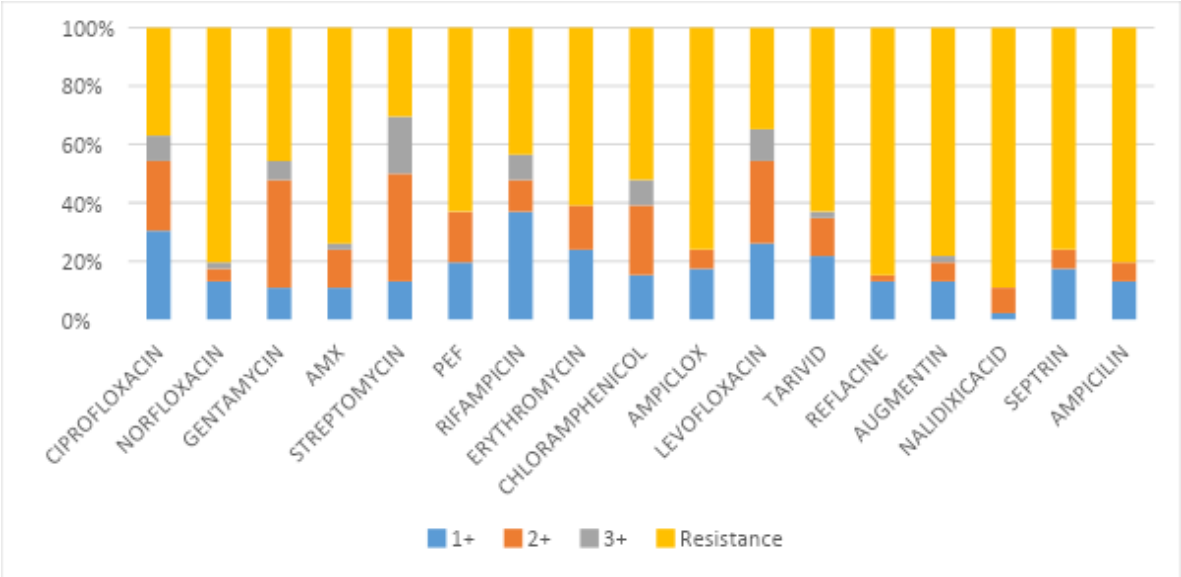

Figure 4. Sensitivity and resistance pattern of *Pseudomonas sp.* to common antibiotics. *(+1= Low sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

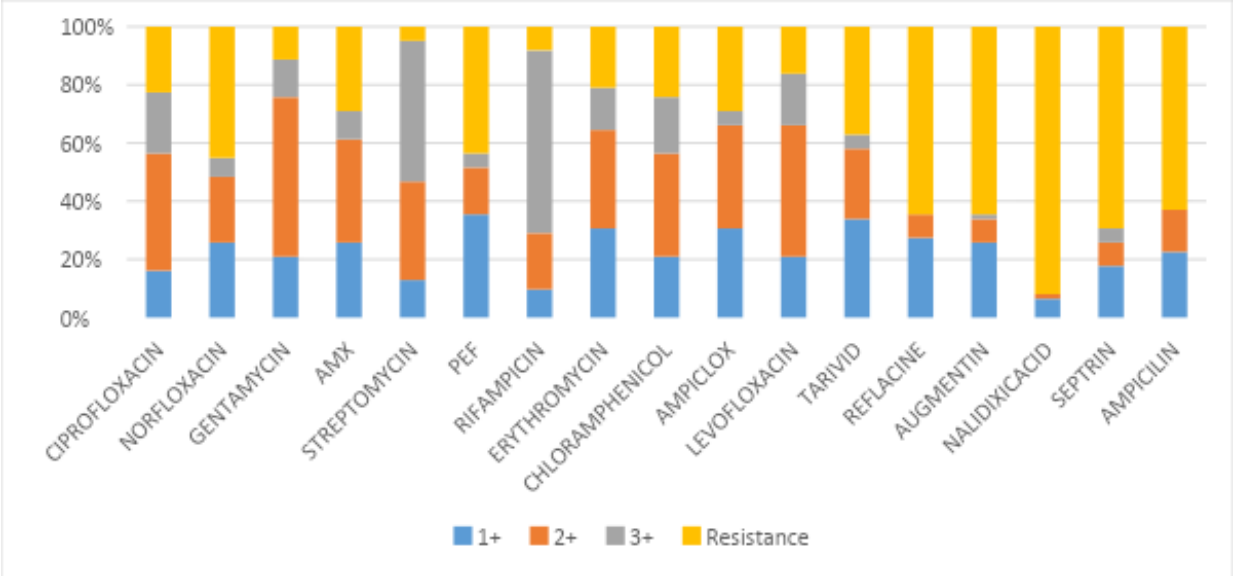

Figure 5. Sensitivity and resistance pattern of *Proteus sp.* to antibiotics. *(+1= Low sensitivity; 2+= Moderate sensitivity; 3+ = Highly sensitive)*

Discussion

The treatment objective of UTIs is essentially to eliminate proliferating bacteria in the urinary tract. This 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 will be less since it is a parenteral medication and is less utilized than readily available oral medications.

In our study, gentamycin 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 be used to 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 uropathens and resistance of 24.8% (78) 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 has the least sensitivity of all the antibiotics, with an M-H of 4.1% and the highest resistance (90.1%)) in our study. 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 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 emphasises the importance of enforcing and strengthening the relevant regulatory bodies to help curtail the indiscriminate use and abuse of antibiotics to combat antibiotic resistance.

C**onclusion**

Our study indicates that levofloxacin had higher sensitivity and lower resistance than ciprofloxacin and ofloxacin. The gram-negative uropathogens are most sensitive and least

resistant to streptomycin, gentamycin and levofloxacin. The uropathogens had 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.

References

1. Reynard J, Brewster S, Biers S. Oxford Handbook of Urology. Third edition. UK: Oxford University Press, 2013: 176-177.

2. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology. 2015 May; 13(5):269-84.

3. Van Schoor J. Urinary tract infections in women. South African Family Practice. 2016 Mar 17; 58:6-10.

4. Raphael JE, Udo K. Bacteriological Spectrum of Urine Culture in patients with Obstructive Uropathy. American Journal of Medical Sciences and Medicine.2022;10(1): 8-22.

5. Najeeb S, Munir T, Rehman S, Hafiz A, Gilani M, Latif M. Comparison of urine dipstick test with conventional urine culture in diagnosis of urinary tract infection. J Coll Physicians Surg Pak. 2015 Feb 1; 25(2):108-114.

6. Price TK, Dune T, Hilt EE, Thomas-White KJ, Kliethermes S, Brincat C, Brubaker L, Wolfe AJ, Mueller ER, Schreckenberger PC. The clinical urine culture: enhanced techniques improve

detection of clinically relevant microorganisms. Journal of clinical microbiology. 2016 May;54(5):1216-1222.

7. Alanazi MQ. Clinical Efficacy and Cost Analysis of Antibiotics for Treatment of Uncomplicated Urinary Tract Infections in the Emergency Department of a Tertiary Hospital in Saudi Arabia. Therapeutics and Clinical Risk Management. 2021;17:1209-1217.

8. Vallejo-Torres L, Pujol M, Shaw E, Wiegand I, Vigo JM, Stoddart M, Grier S et al. Cost of hospitalised patients due to complicated urinary tract infections: a retrospective observational study in countries with a high prevalence of multidrug-resistant Gram-negative bacteria: the COMBACTE-MAGNET, RESCUING study. BMJ Open. 2018 Apr 1;8(4): e020251.

9. Kariuki S, Dougan G. Antibacterial resistance in sub‐Saharan Africa: an underestimated Open emergency. Annals of the New York Academy of Sciences. 2014 Sep;1323(1):43-55.

10. Hooton TM, Stamm WE. Management of acute uncomplicated urinary tract

infection in adults. Med Clin North Am 1991;75:339–57.

11. Odoki M, Almustapha Aliero A, Tibyangye J, et al. Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda. *Int J Microbiol*. 2019; 2019:4246780.

12. Kayima JK, Otieno LS, Tahir A. et al., “Asymptomatic bacteriuria among diabetics attending Kenyatta National Hospital,” East Afr Med J.1996;73(8): 524–526.

13. Moges AF, Genetu A, Mengistu G. Antibiotic sensitivities of common bacterial pathogens in urinary tract infections in Gondar Hospital, Ethiopia. East Afr Med J.2002;79 (3,):140–142.

14. J. Wanyama, “Prevalence, bacteriology and microbial sensitivity patterns among pregnant women with clinically diagnosed urinary tract infections in Mulago Hospital Labour Ward,” Makerere University, Kampala, Uganda, 2003, M.Ed. dissertation of Wanyama. View at: Google Scholar

15. R. Mayanja, C. Kiggundu, D. Kaddu-Mulindwa et al., “The prevalence of asymptomatic bacteriruria and associated factors among women attending antenatal clinics in lower Mulago Hospital,” Makerere University, Kampala, Uganda, 2005, M.Ed. Dissertation of Mayanja.View at: Google Scholar.

16. Oladeinde BH, Omoregie R, Olley M, Anunibe JA. Urinary tract infection in a rural community of Nigeria. North American Journal of medical sciences. 2011 Feb;3(2):75-90.

17. Abdulhadi SK, Yashua AH, Uba A. Organisms causing urinary tract infection in paediatric patients at Murtala Muhammad Specialist Hospital, Kano, Nigeria. International Journal of Biomedical and Health Sciences. 2021;10; 4.

18.Thanassi DG, Saulino ET, Hultgren SJ. The chaperone/usher pathway: a major terminal branch of the general secretory pathway. *Curr Opin Microbiol.* 1998;1:223–231.

19. Piatek R et al. Pilicides inhibit the FGL chaperone/usher assisted biogenesis of the Dr fimbrial polyadhesin from uropathogenic *Escherichia coli*. *BMC Microbiol.* 2013;13:131.

20. Ohnishi Y, Ishikawa J, Hara H, et al. Genome sequence of the streptomycin-producing microorganism Streptomyces griseus IFO 13350. *J Bacteriol*. 2008;190(11):4050-4060.

21. Petroff BP, Lucas FV. Streptomycin in urinary infections. Annals of surgery. 1946;123(5):808.

22. Springer B, Kidan YG, Prammananan T, Ellrott K, Böttger EC, Sander P. Mechanisms of streptomycin resistance: selection of mutations in the 16S rRNA gene conferring resistance. Antimicrobial agents and chemotherapy. 2001 Oct 1;45(10):2877-84.

23. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol*. 2018;4(3):482-501.

24. Goldstein BP. Resistance to rifampicin: a review. The Journal of antibiotics. 2014 Sep;67(9):625-30.

25. Verma E, Chakraborty S, Tiwari B, Mishra AK. Antimicrobial compounds from actinobacteria: synthetic pathways and applications. In New and Future Developments in Microbial Biotechnology and Bioengineering 2018 Jan 1 (pp. 277-295).

26. Graham DB, Tripp J. Ofloxacin. InStatPearls [Internet] 2021 Sep 14. StatPearls Publishing.

27. Goldstein, B. Resistance to rifampicin: a review. J Antibiot. 2014;67: 625–630.

28. Bientinesi R, Murri R, Sacco E. Efficacy and safety of levofloxacin as a treatment for complicated urinary tract infections and pyelonephritis. Expert Opinion on Pharmacotherapy. 2020 Apr 12;21(6):637-44.

29. Bradley JS, Kauffman RE, Balis DA, Duffy CM, Gerbino PG, Maldonado SD, Noel GJ. Assessment of musculoskeletal toxicity 5 years after therapy with levofloxacin. Pediatrics. 2014;134(1):146-53.

30. Ghooi RB, Thatte SM. Inhibition of cell wall synthesis--is this the mechanism of action of penicillins?. *Med Hypotheses*. 1995;44(2):127-131.

31. Hrbacek J, Cermak P, Zachoval R. Current antibiotic resistance trends of uropathogens in Central Europe: Survey from a Tertiary hospital urology department 2011–2019. Antibiotics. 2020 Sep;9(9):630.

32. Nguyen SN, Thi Le HT, Tran TD, Vu LT, Ho TH. Clinical Epidemiology Characteristics and Antibiotic Resistance Associated with Urinary Tract Infections Caused by E. coli. International Journal of Nephrology. 2022 Feb 28;2022.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.