FLUOROQUINOLONES RESISTANCE AMONG BACTERIAL UROPATHOGENS IN A TERTIARY CARE HOSPITAL OF WESTERN NEPAL

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ABSTRACT

Introduction

Urinary tract infections are one of the most common clinical illnesses in developing countries. Most of the infections are treated empirically due to unavailability of laboratory facilities. Fluoroquinolones are frequently used for empirical treatment of UTIs.

Objectives

This study was aimed to determine prevalence and antibiotic resistance pattern of bacterial uropathogens, especially to fluoroquinolones.

Methodology

This hospital based cross sectional study was carried out from September 2019 to January 2020 at Manipal Teaching Hospital, Pokhara, Nepal. A total of 2450 mid stream urine samples were included in this study. Isolation, identification and antibiotic susceptibility testing of the isolates was performed by standard microbiological techniques.

Results

Significant bacteriuria was detected in 258 (10.5%), in significant bacteruria in 614 (25%), growth of multiple organisms in 238 (9.7%) while no growth was recorded in 1340 (54.7%) samples. *E coli* was the commonest isolate followed by *Klebsiella* species. Fluoroquinolones resistance among Gram negative isolates was high. Most of the Gram negative isolates were susceptible to amikacin, gentamicin, nitrofurantoin and imipenem.

Conclusion

High resistance to fluoroquinolones, cotrimoxazole and cephalosporins dictates these to be unsuitable for empirical treatment of UTIs. Amikacin, gentamicin, nitrofurantoin and imipenem were the most effective antibacterials.

KEYWORDS

Antibiotic resistance, bacteriuria, fluoroquinolones, uropathogens, UTIs

INTRODUCTION

Urinary tract infections (UTIs) are one of the commonest clinical conditions affecting all age groups in community as well as hospital settings. Approximately 150 million people suffer from UTIs every year, costing more than 6 billion dollars.[1] It has been estimated that, UTIs result in 8.3 million visits to outpatient clinics, 1 million visits to emergency department and 1,00,000 hospitalization globally.[2] The prevalence is higher in developing countries due to lower level of sanitation and living standard, poor literacy rate, undernourishment and ecological conditions.[3]

Specific antimicrobial agents are essential for successful treatment of UTIs. It has been estimated that, UTIs account for 25% of total antibiotics prescription.[4] In majority of UTIs, broad-spectrum antimicrobials such as cephalosporins, fluoroquinolones and aminoglycosides are prescribed before urine culture and sensitivity test. Fluoroquinolones (ciprofloxacin, norfloxacin and ofloxacin) have been the drugs of choice for empirical treatment of UTIs due to promising effect and ease of administration.[5] Excessive prescription of fluoroquinolones in past few years has led to increased resistance worldwide, particularly among Gram negative organisms.[6,7] This is serious public health issue particularly in the developing countries like Nepal.

Fluoroquinolones are the class of antimicrobial agents in clinical use that directly inhibit the bacterial DNA synthesis. Fluoroquinolones act by inhibiting two different bacterial enzymes, essential for DNA replication, DNA gyrase and topoisomerase IV. This result into bacterial DNA damage and cell death.[8,9]

Due to lack of infrastructure and trained laboratory workers, urine culture and sensitivity is not routinely performed in many laboratories of Nepal. This results into improper diagnosis and forces clinicians to resort to empirical antibiotic treatment. Etiological agents of UTIs and antibiotic susceptibility patterns vary with population, geographical area and existing environmental conditions.[10] The knowledge regarding prevalence of organisms and antibiotics susceptibility trends is essential for prescribing appropriate therapy at local, regional and national level. Therefore, it is important to regularly monitor the susceptibility pattern of pathogens in order to devise appropriate antimicrobial therapy.

This study was planned to investigate the resistance pattern of uropathogens with special attention to fluoroquinolones.

METHODOLOGY

Study design

This hospital based cross sectional study was carried out over a period of five months from September 2019 to January 2020 at Manipal Teaching Hospital, Pokhara, Nepal. Manipal Teaching Hospital is 750 bedded, multispecialty referral center of Western Nepal. Approval from the Institutional Ethical Committee (IRC) of Manipal College of Medical Sciences (MCOMS), Pokhara, Nepal, was obtained before the commencement of the study. Sample size was calculated by formula $N=Z^2pq/d^2$.

Inclusion criteria

All mid stream urine samples from patients suspected of UTI were included in the study.

Exclusion criteria

Patients on antibiotics were excluded from the study. Urine samples collected from catheter bags were also excluded.

Specimen collection, transportation and processing

Mid stream urine samples were collected from out patients and admitted patients of MTH. Samples were immediately transported to the Microbiology laboratory for processing and preserved in refrigerator at 4^0C in case of delay in processing.

Isolation and Identification of Bacterial Isolates

Urine samples were inoculated on MacConkey agar (Hi Media, Mumbai, India) plates using standard calibrated loop. Inoculated plates were incubated at 37°C. Identification of the isolates was performed by standard microbiological techniques such as colony morphology, Gram stain, IMViC test, catalase test and coagulase test.[11]

Antibiotic Susceptibility Test

Antibiotic susceptibility testing of the isolates was performed on Mueller Hinton agar (HI Media, Mumbai, India) by the Kirby–Bauer disc diffusion method.[12] Bacterial isolates showing resistance to at least one agent in three or more antimicrobial categories were labeled as multi drug resistant (MDR).[13] Methicillin resistance in *Staphylococcus aureus* (MRSA) was screened by the cefoxitin (30 μg) disc diffusion method.[12]

Statistical Analysis

The association between variables was explored by the chi-square test using SPSS 11.5. A p value ≤ 0.05 was considered significant.

RESULTS

A total of 2450 urine samples were cultured, 831 (33.9%) from male and 1619 (66.1%) from female patients. The age group of the patients ranged from 2 months to 91 years with mean age 45.68 years (SD: 24.67). Urine culture revealed significant bacteriuria in 258 (10.5%), insignificant bacteruria in 614 (25%), growth of multiple organisms in 238 (9.7%) and no growth in 1340 (54.7%) samples as shown in Figure 1. Significant bacteriuria among females was 66.2% (171/258) and 33.8% (87/258) in males. Details of distribution of bacterial isolates are depicted in Table 1. Majority of the bacterial isolates 87.2% (225/258) were Gram negative and only 12.8% (33/258) were Gram positive. Among the uropathogens, 10.3% (181/1746) isolates were cultured from out patients and 10.9% (77/704) from the admitted patients. Significant bacteriuria was highest 33.3% (86/258) among the old age patients (>60 years) followed by 20.1% (52/258) among 21-30 years patients.

Table 1: Distribution of bacterial isolates from urine samples

Organism	Number (n=258)	Percentage
Escherichia coli	138	53.5
Klebsiella species	41	15.9
Staphylococcus aureus	16	6.2
Enterococcus species	14	5.4
Acinetobacter species	10	3.9
Enterobacter species	09	3.5
Pseudomonas aeruginosa	08	3.2
Citrobacter species	08	3.2
Pseudomonas species	07	2.7
Proteus vulgaris	04	1.5
Staphylococcus saprophyticus	03	1.1

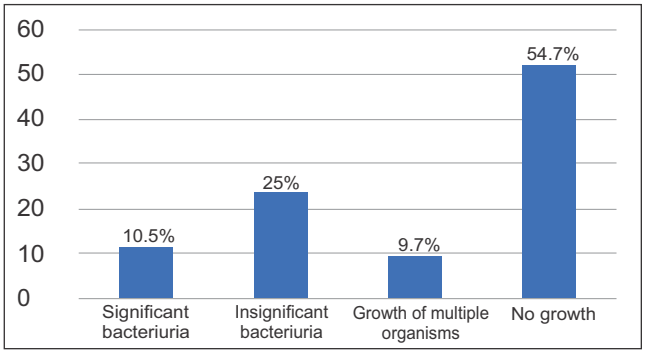

Figure 1: Bacteria growth pattern in urine samples.

Antibiotic susceptibility test results revealed that, most of the Gram negative isolates were susceptible to amikacin, gentamicin, nitrofurantoin and imipenem. Resistance to cotrimoxazole, cefipime, ceftriaxone, ciprofloxacin and norfloxacin was high. Antibiotic resistance pattern of Gram negative isolates is shown in Table 2. Among 225 Gram negative isolates, 38.2% (86/225) were MDR. Most of the Gram positive isolates were susceptible to amikacin, gentamicin and vancomycin. Out of 16 *S. aureus* isolates, 31.2% (5/16) were MRSA. Among 33 Gram positive isolates, 18.2% (6/33) were MDR. Antibiotic resistance pattern of Gram positive isolates is shown in Table 3.

Fluoroquinolones resistance was highest among isolates from >60 years age group. The age wise distribution of the isolates and fluoroquinolones resistance is shown in Table 4. Fluoroquinolones resistance among inpatient isolates was 63.6% (49/77) which is higher than outpatient isolates 43% (78/181). This difference was statistically significant (P value <0.05). Among the fluoroquinolones resistant uropathogens (n=127), majority of the isolates (71/127) were from the referred patients.

Table 3: Antibiotic resistance pattern of Gram positive bacteria

Organism	Antibiotics (percentage resistance)								
	AK	CIP	COT	CTR	GEN	CX	ERY	CD	VA
S. aureus (n=16)	0	25	25	18.7	0	31.2	31.2	31.2	0
S. saprophyticus (n=3)	0	0	0	0	0	0	33.3	0	0
Enterococcus spp (n=14)	0	64.3	—	—	14.3	—	64.3	—	0

CX: Cefoxitin, ERY: Erythromycin, CD: Clindamycin, VA: Vancomycin

Table 4: Age wise distribution of the isolates with fluoroquinolones resistance

Age group	Total number of isolates n=258 (%)	Fluoroquinolones resistant isolates (%)
2 months-10 years	19 (7.3)	9 (47.3)
11 years - 20 years	21 (8.1)	8 (38)
21 years - 30 years	52 (20.1)	18 (34.6)
31 years - 40 years	31 (12)	12 (38.7)
41 years - 50 years	22 (8.5)	11 (50)
51 years - 60 years	27 (10.4)	13 (48.1)
>60 years	86 (33.3)	56 (65.1)

DISCUSSION

Urinary tract infections are one of the most commonly diagnosed conditions in the laboratory. Antimicrobial resistance pattern of uropathogens has changed over a period of time and increasing resistance has been reported globally. Drug resistance among uropathogens is often associated with increased cost, poor outcome and recurrence. The contributory factors include extensive use of antimicrobial agents, hospitalization, diagnostic and therapeutic procedures. Periodic surveillance of antibiogram of uropathogens at local, regional and national level is necessary to understand the dynamics and devise antibiotic policy/stewardship.

This study highlights the distribution and antibiotic resistance patterns of bacterial uropathogens. Significant bacteriuria was detected in 10.5% (258/2450) cases. Prevalence of significant bacteriuria depends on various factors like age group, socioeconomic status, antimicrobial therapy, laboratory techniques of isolation etc. Baral *et al*

Table 2: Antibiotic resistance pattern of Gram negative bacteria

Organism	Antibiotics (percentage resistance)									
	AK	CIP	COT	CTR	GEN	CFM	CFS	NX	NIT	IPM
E coli (n=138)	10.1	60.1	39.1	52.9	15.9	58.7	26.1	59.4	8.7	2.2
Klebsiella spp (n=41)	14.6	41.4	31.7	39	14.6	58.5	21.9	41.4	29.2	4.8
Acinetobacter spp (n=10)	30	30	30	—	30	30	—	—	—	0
Pseudomonas aeruginosa (n=8)	0	37.5	37.5	—	0	37.5	—	—	—	0
Pseudomonas spp (n=7)	0	42.8	14.3	—	14.3	14.3	—	—	—	0
Enterobacter spp (n=9)	0	33.3	22.2	22.2	22.2	33.3	33.3	33.3	33.3	0
Citrobacter spp (n=8)	0	25	25	25	0	25	25	25	25	0
Proteus vulgaris (n=4)	0	0	0	0	0	0	0	0	—	0

AK: Amikacin, CIP: Ciprofloxacin, COT: Cotrimoxazole, CTR: Ceftriaxone, GEN: Gentamicin, CFM: Cefipime, CFS: Cefaperazone sulbactum, NIT: Nitrofutantoin, NX: Norfloxacin, IPM: Imipenem

from Kathmandu, Nepal, reported 30.8% significant bacteriuria which was higher than our findings.[14] Manipal Teaching Hospital being referral hospital, many patients are referred from other hospitals and clinics after primary treatment. This could be associated with lower percentage of significant bacteriuria in our study as previous antibiotic treatment has direct impact on bacterial count. Female patients comprised 66.2% of total cases of significant bacteriuria, reflecting higher risk of UTIs among female. The higher prevalence among females is due to the anatomical structure and lack of secretions from prostate which has bactericidal property.[15,16] Similar findings have been reported by studies from Nepal and other countries.[14,17] The commonest uropathogen in our study as reported elsewhere was *E coli* (53.5%) followed by *Klebsiella* species (15.9%).[17,18] *E coli* and *Klebsiella* species together accounted for 69.4% of all uropathogens. In our study, highest percentage of uropathogens was recorded among older age group (>60 years) population followed by reproductive age group of 21-40 years. Yadav *et al*, from Nepal reported highest percentage of uropathogens among reproductive age group of population.[19]

Increasing drug resistance among *E coli* and *Klebsiella* species is serious issue which requires continuous surveillance and monitoring. The antibiotic susceptibility test results revealed that, Gram negative isolates were highly sensitive to gentamicin, amikacin, nitrofurantoin and imipenem. Antibiotics like cotrimoxazole, cifipime, ceftriaxone, ciprofloxacin and norfolxacin were less effective. Increasing resistance of uropathogens to fluoroquinolones is alarming. Fluoroquinolones like ciprofloxacin, ofloxacin have been extensively used in past few years not only to treat UTIs but wide variety of other bacterial infections. In our study, resistance of *E coli* to fluoroquinolones was high (ciprofloxacin 60.1% and norfloxacin 59.4%) indicating these drugs are not suitable for empirical treatment of UTIs. Baral *et al*, reported lower resistance (36%) of *E coli* to fluoroquinolones[14] while Sherchan *et al*, reported higher resistance (68.57%).[20] This reflects that, resistance pattern of the uropathogens vary greatly in different settings and findings of particular setting cannot be generalized. Based on the findings of our study, nitrofurantoin can be an alternative drug for empirical treatment of UTIs among outpatients as well as inpatients. Nitrofurantoin can be administered orally and is highly concentrated in urine; it may therefore be the most appropriate agent for empirical use in uncomplicated UTIs.

Our study highlights the increasing number of multidrug resistant uropathogens among outpatients and inpatients. Among Gram negative bacteria, 27% (49/181) were MDR in outpatients and 48% (37/77) from admitted patients. This difference was statistically significant (P value <0.005). The majority of MDR isolates were resistant to commonly prescribed antibiotics like cotrimoxazole, fluoroquinolones and cephalosporins. Association of MDR strains with UTIs may result into treatment failure and recurrence. In our study, 38.4% *E coli* isolates were MDR. Findings of our study are comparable with study by Baral *et al*, reported 38.2% MDR *E coli* isolates from urine samples.[14] High incidence of MDR among the commonest uropathogen like *E coli* results into poor outcome with traditional empirical therapy. High resistance to broad spectrum antibiotics like cephalosporins and fluoroquinolones with emergence of MDR strains necessitates the use of carbapenems as a drug of choice. However, emergence of imipenem resistant isolates limits the treatment options. In our study, 2.2% isolates of *E coli* and 4.8% *Klebsiella* species were resistant to imipenem. Another study from Nepal, reported emergence of imipenem resistant *E coli*.[19,20] In developing countries like Nepal, improper use and easy access of antibiotics with poor monitoring of antibiotic resistance pattern, results in high percentage of resistance to commonly prescribed drugs.

Staphylococcus aureus was the most common Gram positive bacteria and 31.2% isolates were MRSA. Another study from Nepal reported higher percentage of MRSA (47.61%) among uropathogens.[18] Similar studies from India reported higher percentage of MRSA as compared to our study.[21,22] Lower percentage of MRSA in our study could be associated with higher number of community acquired *S. aureus* isolates. Resistance to vancomycin was not recorded.

CONCLUSION

High prevalence of fluoroquinolones resistance among uropathogens was observed. Fluoroquinolones, therefore are not suitable options for empirical treatment of UTIs among the patients of tertiary care hospital. Based on susceptibility test results, amikacin, gentamicin and nitrofurantoin provide better treatment options in resource limited settings. Regular surveillance of MDR isolates in different regions would help to understand the changing trends of resistance pattern of uropathogens and the successful management.

RECOMMENDATIONS

Fluoroquinolones should not be used for empirical treatment of UTIs in Western region of Nepal. Amikacin, gentamicin and nitrofurantoin could be used for empirical treatment.

LIMITATION OF THE STUDY

Minimal inhibitory concentration of the fluoroquinolones was not performed.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

FINANCIAL DISCLOSURE

None

REFERENCES

1. Gonzalez CM, Schaeffer AJ. Treatment of urinary tract infection: what's old, what's new, and what works. World Journal of Urology. 1999; 17(6):372–382. PMID: 10654368
2. Naber KG, Schito G, Botto H, Palou J, Mazzei T. European Urology. 2008; 54(5):1164-1175. DOI: 10.1016/j.eururo.2008.05.010
3. Cheesbrough M. District laboratory practice in tropical countries. Cambridge Univ press. 2000; 2: 125-137.
4. Hooton TM. Uncomplicated urinary tract infection. N Engl J Med. 2012; 366: 1028-37. DOI: 10.1056/NEJMcp1104429.
5. Shariff VAA, Shenoy MS, Yadav T, Radhakrishna M. The antibiotic susceptibility patterns of uropathogenic Escherichia coli, with special reference to the fluoroquinolones. J Clin Diagn Res. 2013; 7:1027-30. DOI: 10.7860/JCDR/2013/4917.3038
6. Acar JF, Goldstein FW. Trends in bacterial resistance to fluoroquinolones. Clin Infect Dis. 1997; 24 (Suppl 1): 67-73. PMID: 8994781
7. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis. 2006; 6: 629-40. DOI: 10.1016/S1473-3099(06)70599-0
8. Drlica K, Malik M, Kerns RJ, Zhao X. Quinolone-mediated bacterial death. Antimicrobial Agents and Chemotherapy. 2008; 52(2):385–392. DOI: 10.1128/AAC.01617-06
9. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiology and Molecular Biology Reviews. 1997; 61(3):377–392. PMID:232616
10. Saffar MJ, Enayti AA, Abdolla IA, Razai MS, Saffar H. Antibacterial susceptibility of uropathogens in 3 hospitals, Sari, Iran, 2002-2003. Eastern Mediterranean Health Journal. 2008; 14(3):556-563. PMID: 18720619
11. Harley JP, Prescott LM. Laboratory exercises in Microbiology, 5th edition. Mc-Graw-Hill Publishers. 2002; 37-237.
12. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, Twenty- Second Informational Supplement, CLSI, Document M100–S27, CLSI, Wayne, PA, USA, 2017.
13. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske G, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18:268–81. DOI: 10.1111/j.1469-0691.2011.03570
14. Baral P, Neupane S, Marasini BP, Ghimire KR, Lekhak B, Shrestha B. High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. BMC Research Notes. 2012, 5:38. DOI: 10.1186/1756-0500-5-38
15. Ochei J, and Kolhatkar A. Diagnosis of infection by specific anatomic sites/antimicrobial susceptibility tests in Medical Laboratory Science Theory and Practice reprint, 6th edition, McGraw-Hill, New Delhi, India. 2007; 615–643.
16. Aiyegoro OA, Igbinosa OO, Ogunmwonyi IN, Odjadjaro E, Igbinosa OE, Okoh AI. Incidence of urinary tract infections (UTI) among children and adolescents in Ile-Ife, Nigeria. African Journal of Microbiological Research. 2007; 1:13–19. DOI: 10.5897/ 311673210765
17. Pradhan B, Pradhan SB. Prevalence of Urinary Tract Infection and Antibiotic Susceptibility Pattern to Urinary Pathogens in Kathmandu Medical College and Teaching Hospital, Duwakot. BJHS. 2017; 2 (1):134-137. DOI: http://dx.doi.org/10.3126/bjhs.v2i1.17290
18. Singh VK, Tuladhar R, Chaudhary MK. Beta Lactamase Producing Escherichia coli, Klebsiella pneumoniae and Methicillin Resistant Staphylococcus aureus among Uropathogens. Nepal Journal of Science and Technology. 2015; 16(1):105-112. DOI: http://dx.doi.org/10.3126/njst.v16i1.14364
19. Yadav K, Prakash S. Screening of ESBL Producing Multidrug Resistant E coli from Urinary Tract Infection Suspected Cases in Southern Terai of Nepal. J Infect Dis Diagn. 2017; 2(2):116. DOI: 10.4172/2576-389X.1000116
20. Sherchan JB, Gurung P, Karkee P, Ranabhat N, Shrestha N, Ohara H. Microbiological and Clinical Profile of Uropathogenic Escherichia coli Isolates in Kathmandu University Hospital. J Nepal Health Res Counc. 2016; 14(32):33-38. PMID: 27426709
21. Dalela G, Gupta S, Jain DK, Mehta P. Antibiotic Resistance Pattern in Uropathogens at a Tertiary Care Hospital at Jhalawar with Special Reference to ESBL, AmpC b-Lactamase and MRSA Production. Journal of Clinical and Diagnostic Research. 2012; 6(4): 645-651. DOI: /2012/4201:0025
22. Aggarwal R, Goel N, Chaudhary U, Goyal S, Dahiya S. Prevalence of MRSA as uropathogen in a teaching tertiary care hospital of North India. Int. J. Pharm. & Bio. Sc. 2013; 2(2). DOI: 2279-0853