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Amrit Research Journal December 2024, Vol. 5, Issue 1, 114-121
ISSN: 2738-9553
RESEARCH ARTICLE

Prevalence and Antibiotic Susceptibility Pattern Of Uropathogens From Urinary Tract Infection Suspected Patients Visiting Tertiary Care Hospital of Nepal

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(Received: August 13, 2024; Received in Revised form: October 22, 2024; Accepted: October 29, 2024; Available online)

DOI: https://doi.org/10.3126/arj.v5i1.73562

Highlights:

- Dominance of *Escherichia coli* was observed amongst other uropathogens.
- *Klebsiella pneumoniae* showed higher antibiotic resistance to all major antibiotics class.
- Higher resistance towards cephalosporin was displayed by most of the uropathogens.
- WHONET software ((version 23.8.14) was used to screen the high priority pathogens.
- High-priority pathogens were observed in abundance within uropathogens.

Abstract

Antibiotic resistant uropathogens is a significant problem in all patient, leading to high morbidity, poor quality of life and a limited life expectancy thus situation analysis of those pathogens is necessary. Hence, this study aims to detect uropathogens and evaluate their current antibiotic resistant pattern. Mid-stream urine samples were cultured for bacterial isolation and isolates were identified using biochemical tests. Antibiotic susceptibility testing was done using disc diffusion method. The results were analyzed using statistical software. Out of 1784 suspected cases, 182 urine samples showed significant growth. Among uropathogens, Escherichia coli was predominant followed by Klebsiella pneumoniae, Proteus mirabilis Staphylococcus aureus, Pseudomonas aeruginosa, Morganella morganii, and Acinetobacter spp. The antibiotic-resistant pattern was observed highest among K. pneumoniae and E. coli. Also, higher resistance was observed towards Cephalexin (70.3%), followed by Ceftazidime (70.2%) and Nalidixic acid (67.6%) and Cefixime (62.5%). And, a total of 46.7% (n=85) multidrug resistant isolates were detected of which 17 were identified as high risk clones. In conclusion, antimicrobial resistance among urine isolates, particularly E. coli and K. pneumoniae seem to be emerging. Therefore, antimicrobial resistance trend analysis exploring both phenotypic and molecular techniques would be helpful in intervening in antimicrobial resistance development and spread.

Keywords: *Uropathogens, Antimicrobial resistance, Escherichia coli, Klebsiella pneumoniae, High-priority pathogen*

Introduction

Urinary tract infections (UTIs) caused by uropathogens are the leading cause of mortality and growing hospital expenditure worldwide [1]. These infections range from regular cases to recurrence cases [2-3]. Compared to other bacterial infections, UTIs are the most typical bacterial infection, and they can occur in both community and hospital settings [3]. The predominance of

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UTIs over other infections is due to a combination of factors, including hereditary factors, biological factors, and underlying disorders [2, 4]. According to reports, there were more than 404.6 million cases reported in 2019 worldwide, and their global burden is estimated to be rising each year [2]. Several bacteria contribute to UTIs, but the most common uropathogens are Gram-negative bacteria, particularly *E. coli*, which accounts for the majority of the cases in different settings [3-6]. While the less common Gram-negative bacteria are *Klebsiella* spp., *Pseudomonas aeruginosa,* and *Proteus* spp. even some of the Gram-positive bacteria, like *Staphylococcus saprophyticus* and *Streptococcus* spp. has been reported as the causative agent of UTI [4]. Commonly, different antibiotics are used for treating UTIs based on their susceptibility to respective uropathogens. But the emergence of resistance to these antibiotics, especially to multiple classes, has questioned their efficacy and limited the options for disease treatment. This rising antimicrobial resistance (AMR) among uropathogens is a matter of global concern, but its burden is felt greatly within low- and middle-income countries like Nepal [1]. The practice of continuous and rampant use of antibiotics has led to this menace [7]. Numerous studies from Nepal indicate a worrying elevation in the AMR [7-9], especially among uropathogens [10–17]. Studies show that, within uropathogens, susceptibility to Penicillin or first-line antibiotics is a rare instance nowadays, while resistance to Cephalosporins, Aminoglycosides, Quinolones and Carbapenem is becoming more common. Such an upsurge in resistance towards important classes of drugs is a worrisome situation, particularly for multidrug-resistant (MDR) strains [10–14, 16]. Besides, the most nuisance isolates, particularly the extended spectrum beta-lactamase (ESBL) [11–13, 16] and carbapenem resistance enterobacteriaceae (CRE) [8, 17], have also been reported within uropathogens in Nepal. These ESBL and CRE bacteria are difficult to treat and are easily transmitted via horizontal gene transfer [11–13, 16–17], so timely detection and reporting are essential in intervening in the emergence and spread of AMR [7-8, 15]. Although several reports are available on the antimicrobial resistance of uropathogens in Nepal, the continuous trend analysis of their resistance patterns within different groups is always important. This situation analysis can be used as a guideline report for clinicians and policymakers to understand the trend of AMR and plan for the control and treatment of AMR infections.

Materials and Methods

Bacteria isolation and identification

A cross-sectional study was carried out from June 2020 to March 2021 in the Green City Hospital, Basundhara, Kathmandu. Mid-stream clean void urine samples from UTI-suspected patients were included in this study. Samples were cultured using the conventional streaking method, and those with significant growth (≥10 5 CFU/ml) were selected [18]. The identification of bacterial isolates was done using standard microbiological techniques, which included studying colony characteristics, staining reactions, and biochemical tests [19].

Antimicrobial susceptibility testing

The antibiotic susceptibility test of the isolates was performed by the Kirby-Bauer disk diffusion method on Mueller Hinton agar (MHA) as per the Clinical Laboratory Standards (CLSI) recommendations and interpretation criteria [20]. The antibiotic discs from Hi-Media Laboratories Private Limited were used, and they were from 10 different classes of antibiotic. The classes were aminoglycosides (Amikacin AK_30µg ; Gentamycin GEN_10µg), cephalosporin (Cefotaxime CTX_30µg; Ceftazidime CAZ_30µg; Cephalexin CN_30µg; Cefixime CFX_30µg), penicillin (Amoxicillin AMX_10µg; Piperacillin PI_30µg), penicillin/beta lactamase inhibitor combination (Piperacillin-tazobactam PTZ_110µg; Amoxicillin-Clavunate AMC_30µg), tetracycline (Tigecycline TGC_30µg), quinolone (Nalidixic acid NA_30µg; fluoroquinolone (Ciprofloxacin CIP_5µg; Norfloxacin NX_10µg), carbapenem (Imipenem IMP_10µg; Meropenem MEM_10µg), Nitrofurans (Nitrofurantoin NIT_300µg), Lipopeptide (Polymixin B PB_300µg; Colistin CL_10µg) and sulfonamides (Sulfamethoxazole-trimethoprim COT_25µg).

Statistical Analysis

Statistical analysis was done using IBM SPSS Statistics (Version 20) and WHONET_2023 (version 23.8.14) software.

Results and Discussion

Demographic distribution

Of the total 1784 urine samples tested, 182 samples (10.25%) were found to be urine culture positive with significant growth, which is in conjunction with other studies [13–15]. Based on demographic characteristics (Table 1), the prevalence indicates that

the UTI is high among the 16–30-year-old age group, which is similar to earlier reports [13, 17, 21, 22]. Since this age group is sexually more active [4], the high prevalence within this group is relatively understandable. Further, this study showed female patients had a higher UTI prevalence than their male counterparts, and this proportional difference may be due to the fact that females are more susceptible to UTI due to many predisposing factors [3, 4]. Moreover, evidently, many studies from Nepal [15–17] and other parts of the world [21–22, 24–25] suggested a similar high UTI prevalence among females.

Microbial diversity

Based on Gram staining, 95.1% (n = 73) were Gram-negative and 4.9% (n = 9) were Gram-positive bacteria. In terms of microbial isolates, a diverse group of microorganisms were isolated, and the Enterobacteriaceae family was the major group among all (Table 1). As evident from earlier reports [10–17, 21–25], *E. coli* is frequently found in urine samples and is the most predominant strain. A similar higher proportion of *E. coli* strains (n = 123, 66.85%) were observed in this study. This dominance of *E. coli* was observed in all demographic categories, irrespective of the patients' age and gender (Table 1). The high occurrence of *E. coli* can be explained in terms of their ubiquitous nature [3–4]. Though commensal in nature, *E. coli* has a high potential to turn into a pathogenic strain and cause a variety of infections [3, 6]. And this conversion is contributed by sets of complementary virulence factors that help them survive and cause disease [6]. Beyond the pathogenic *E. coli*, *K. pneumoniae* was the second most abundant species, followed by the less common uropathogens like *Proteus mirabilis, Pseudomonas aeruginosa, Morganella morgani, and Acinetobacter* spp. *Staphylococcus aureus*. As per earlier studies, these less common uropathogens are generally associated with specific clinical conditions, particularly complicated infection cases [3]. And in this study, urine samples from all types of patients, ranging from outpatients to ICU patients, have been included, so the occurrence of less common uropathogens is apparent.

Table 1: Bacterial etiology of UTI based on demographic characteristics of study participants (N =182)

	Category	Isolate Count	*Escherichia coli* (N=123)	*K. pneumoniae* (N=33)	*Proteus mirabilis* (N=7)	*Acinetobacter* spp. (N=1)	*Morganella morgana* (N=3)	*Staphylococcus aureus* (N=9)	*Pseudomonas aeruginosa* (N=6)
Gender	Female	**132**	95(72.0%)	18(13.6%)	6(4.5%)	1(0.8%)	3(2.3%)	7(5.3%)	2(1.5%)
	Male	**50**	28(56.0%)	15(30.0%)	1(2.0%)	0	0	2(4.0%)	4(8.0%)
Age group	0-15	**2**	1(50%)	0	0	0	0	0	1(50%)
	16-30	**66**	47(71.2%)	7(10.6%)	5 (7.6%)	1(1.5%)	1(1.5%)	4(6.1%)	1(1.5%)
	31-45	**38**	26(68.4%)	8(21.1%)	1(2.6%)	0	1(2.6%)	2(5.3%)	0
	46-60	**40**	23(57.5%)	10(25.0%)	1(2.5%)	0	1(2.5%)	3(7.5%)	2(5.0%)
	60 above	**36**	26(72.2%)	8(22.2%)	0	0	0	0	2(5.6%)

Antibiotic susceptibility pattern

In this study, higher resistance was observed towards Cephalexin while lowest resistance towards Tigecycline (Figure 1). Based on the class of antibiotic, resistance towards Cephalosporins was apparently high compared to others.

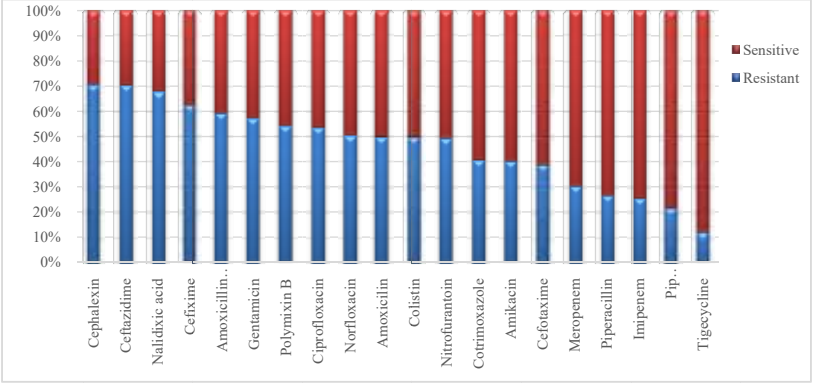

Fig 1. Antibiotic susceptibility pattern of uropathogens towards common antimicrobials.

In the case of specific isolates, the antibiotic susceptibility was different within and among the different strains. The burden of antibiotic resistance was observed in higher proportions within *E. coli* strains, followed by *K. pneumonia* (Table 2).

Table 2. Resistant percentage of strains towards different antibiotics

1 ***Table 2.*** *Resistant percentage of strains towards different antibiotics*

Antibiotic used	Number of isolatetested	*Escherichia coli*		*Klebsiella pneumoniae*		*Proteus mirabilis*		*Acinetobacter* spp.		*Morganella morganii*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
AK	127	36	41.9%	9	42.9%	1	14.3%	1	100%	1	33.3%	3	42.9%	0	-
AMC	27	12	63.2%	3	100%	0	-	0	-	0	-	1	25.0%	0	-
AMX	22	6	40.0%	1	100%	0	-	0	-	0	-	3	60.0%	1	100%
CAZ	47	16	55.2%	10	90.9%	2	100%	0	-	0	-	2	100%	3	100%
CFM	56	20	52.6%	9	75.0%	0	-	1	100%	0	-	3	100%	2	100%
CIP	73	25	52.1%	9	64.3%	1	100%	0	-	0	-	3	75.0%	1	33.3%
CL	10	1	100%	3	50%	0	-	0	-	0	-	0	-	1	50%
CN	64	29	64.4%	8	100%	3	100%	0	-	0	-	3	60.0%	2	100%
COT	32	8	34.8%	1	25.0%	1	100%	0	-	1	100%	1	50%	1	100%
CTX	62	19	42.2%	3	37.5%	0	-	0	-	0	-	0	-	2	100%
GEN	21	10	62.5%	2	50%	0	-	0	-	0	-	0	-	0	-
IMP	51	5	17.2%	5	31.3%	0	-	0	-	0	-	1	100%	2	50%
MEM	69	5	13.5%	9	45.0%	0	-	0	-	0	-	4	66.7%	3	100%
NA	136	63	68.5%	13	48.1%	5	100%	1	100%	2	66.7%	5	100%	3	100%
NIT	176	45	37.2%	23	71.9%	4	57.1%	0	-	3	100%	7	87.5%	5	100%
NX	111	36	46.2%	12	63.2%	4	80.0%	1	100%	0	-	3	75.0%	0	-
PB	74	28	60.9%	8	47.1%	1	25.0%	0	-	2	100%	1	33.3%	0	-
PI	15	2	22.2%	2	50%	0	-	0	-	0	-	0	-	0	-
PTZ	140	17	17.2%	10	41.7%	0	-	0	-	0	-	2	25.0%	1	25.0%
TGC	66	4	8.9%	2	14.3%	0	-	0	-	0	-	1	50%	1	33.3%

(Note: AK=Amikacin, GEN=Gentamycin, CTX=Cefotaxime, CAZ=Ceftazidime, CN=Cephalexin, CFX=Cefixime, AMX=Amoxicillin, PI=Piperacillin, PTZ=Piperacillin-Tazobactam, AMC=Amoxicillin-Clavunate, TGC=Tigecycline, NA=Nalidixic acid, CIP=Ciprofloxacin, NX=Norfloxacin, IMP=Imipenem, MEM=Meropenem, NIT=Nitrofurantoin, PB=Polymixin_B, CL=Colistin, and COT=Sulfamethoxazole-trimethoprim).

At species level, *E. coli* showed high resistance to Cephalosporins (Table 2). Similar Cephalosporins resistance was reported in the findings of Parajuli et al. (2017) and Raya et al. (2020). In the case of *K. pneumoniae*, higher resistance was observed within Penicillin and Cephalosporins classes of drugs (Table 2). A similar finding of Penicillin resistance was reported by Ahmed et al. (2019), while Cephalosporin resistance was reported by Raya et al. (2020). As for the other uncommon uropathogens, *S. aureus* showed higher resistance towards Cephalosporins and carbapenem, while Pseudomonas aeruginosa showed high resistance towards all the major classes of antibiotics (Table 2). Moreover, *E. coli* as well as *K. pneumoniae* strains exhibiting resistance to Carbapenem (Meropenem and Imipenem) were also observed in this study. In Nepal, several studies have already reported the occurrence of such Carbapenem-resistant strain among uropathogens [17] and due to limitation in their treatment options, they are a great challenge for clinicians.

In this study, out of total isolates, 96.7% (n = 176) showed resistance to at least one antibiotic, while 46.7% of isolates were identified as MDR. A similar finding was reported by Ghimire et al. (2021), while other studies reported a comparatively much higher prevalence of MDR from Nepal [10, 13–14, 16]. The distribution of MDR and non-MDR within different category was not statistically significant ($p>0.05$) (Table 3). Based on strain type (Table 3), more than half of the MDR was *E. coli* (n = 50, 58.8%), which indicates that the AMR among *E. coli* is on the rise. Besides, a similar high prevalence of MDR uropathogenic *E. coli* has been regularly reported in Nepal [13–16] as well as in other parts of the world [5, 21, 23].

Table 3: Distribution of MDR and Non-MDR isolates within different category (N=182)

Category		MDR (N=85)		Non-MDR (N=97)	
Count		%	Count	%	
Organism Isolated	*Escherichia coli*	50	58.8%	73	75.3%
	Klebsiella pneumoniae	18	21.2%	15	15.5%
	Proteus mirabilis	2	2.4%	5	5.2%
	Acinetobacter spp.	1	1.2%	0	0.0%
	Morganella morgani	2	2.4%	1	1.0%
	Staphylococcus aureus	7	8.2%	2	2.1%
	Pseudomonas aeruginosa	5	5.9%	1	1.0%

Moreover, the WHONET analysis showed that 39 (21.43%) isolates could be possible extensive drug resistance (XDR), which is noteworthy. Based on the WHO priority pathogen list, 17 were screened as high-priority isolates as they were resistant to Carbapenem and 91 were screened as medium-priority isolates. Of the 17 high-priority isolates, 8 (47.06%) were *E. coli* and 9 (52.94%) were *K. pneumoniae* (Table 4).

Since resistance to the major classes of drugs can interfere with the treatment of the infection and lead to treatment failures, so the prevalence of MDR should not be disregarded. Moreover, antibiotic resistance can be easily transferred and disseminated among the bacterial population [5], so both the emergence and spread of AMR should be checked and controlled.

Table 4: Antibiotic resistant profile of high priority isolates (N=17) according to WHONET 2023 analysis

Organism	Count	Resistant profile	MDR	Possible XDR	Priority
E. coli	1	CAZ-PTZ-AMX-IPM-LE	Yes	Yes	High
E. coli	1	AK-CAZ-MEM-NIT-PTZ- IPM	Yes	Yes	High
E. coli	1	AK-CFM-CIP-MEM-NA-NIT-PTZ-IMP-LE -CL	Yes	Yes	High
E. coli	1	AK-MEM-NIT-LE	Yes	Yes	High
E. coli	1	CFM-CIP-NA-PTZ-IPM-NX	Yes	Yes	High
E. coli	1	CIP-MEM-NA-NIT-PTZ-COT-NX	Yes	Yes	High
E. coli	1	AK-CAZ-CIP-NA-NIT-PTZ-CTX-IMP-NX	Yes	Yes	High
E. coli	1	MEM-NIT-PTZ-CTX-LE	Yes	No	High
K. pneumoniae	1	AK-CAZ-MEM-NA-NIT-PTZ-PI-NX-LE	Yes	Yes	High
K. pneumoniae	2	AK-CFM-CAZ-CIP-MEM-NA-NIT-PTZ-IMP-CL	Yes	Yes	High

K. pneumoniae	1	AK-CAZ-CIP-MEM-NIT-IMP-PB-CL	Yes	Yes	High
K .pneumoniae	1	CAZ-CIP-MEM-NIT-PTZ-LE	Yes	Yes	High
K. pneumoniae	1	AK-CFM-MEM-NA-NIT-PTZ-IMP-TGC-NX	Yes	Yes	High
K. pneumoniae	1	AMC-CFM-MEM-NA- NIT-IMP-TGC-NX-LE	Yes	Yes	High
K. pneumoniae	1	AK-CIP-MEM-NA-NIT-PTZ-NX	Yes	Yes	High
K. pneumoniae	1	CFM-MEM-NA-NIT-PTZ-NX	Yes	Yes	High

(Note: AK=Amikacin, GEN=Gentamycin, CTX=Cefotaxime, CAZ=Ceftazidime, CN=Cephalexin, CFX=Cefixime, AMX=Amoxicillin, PI=Piperacillin, PTZ=Piperacillin-Tazobactam, AMC=Amoxicillin-Clavunate, TGC=Tigecycline, NA=Nalidixic acid, CIP=Ciprofloxacin, NX=Norfloxacin, IMP=Imipenem, MEM=Meropenem, NIT=Nitrofurantoin, PB=Polymixin_B, CL=Colistin, and COT=Sulfamethoxazole-trimethoprim).

Evidently, in Nepal, antibiotic resistance among the bacteria is the consequence of uncontrolled prescription, over-the-counter use, and a lack of a proper surveillance program for AMR [7]. Thus, timely detection tools, a continuous monitoring program, and controlled usage of antibiotics may be pivotal in regulating the emergence and spread of such isolates.

Conclusions

In conclusion, the frequency of high-priority pathogens particularly carbapenem resistant *E. coli* and *K. pneumoniae,* was found to be higher in this study. These routine uropathogenic strains showing resistance to multiple antibiotics that belong to the major class, is a worrisome situation. Therefore, bacterial AMR, which is a global threat, should be the center of attention in the public health field and a comprehensive study is required. And this kind of research focusing on uropathogens that are the key players in AMR evolution and spread is absolutely necessary to ensure proper intervention in AMR emergence and spread.

Conflict of Interests

All the authors declare that there are no financial or non-financial competing interest between the authors or with the funding agency.

Authors' Contributions

Munal Subedi- funding acquisition and manuscript reviewing; Sony Thapaliya- Lab investigation and manuscript reviewing; Suchitra Thapa- conceptualization, funding acquisition, sampling, monitoring and supervision, data analysis, data interpretation, draft manuscript preparation, reviewing and editing.

Consent and Ethics Approval

Written consent was taken during sampling, from each participant after explaining the aim and purpose of the research to them. Ethical approval from concerned authority for clinical samples was taken before proceeding and the approval number is NHRC reg.no. 169/2020.

Acknowledgement

All authors are grateful to UGC Nepal as this study was funded by University Grant Commission, Kathmandu, Nepal under the small research and development innovation research grant number No: SRDI-75/76-S&T-11. Additionally, all authors are highly indebted to the staff of pathology department in Green City Hospital for their support. The authors are also thankful to Mr. Sandesh Rimal for his help in sample collection even during pandemic situation.

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