

# Antibiotic Susceptibility Pattern of Bacteria Causing Urinary Tract Infection at Nepalgunj Medical College

Gurung K<sup>1</sup>, KC R<sup>2</sup>, Patel I<sup>2</sup>, Neupane DR<sup>1</sup>, Pokhrel S<sup>1</sup>, Gurung S<sup>3</sup>

## ABSTRACT

**Introduction:** Urinary Tract Infection is one of the most common infectious diseases affecting people of all age groups. Resistance to antibiotics is highly prevalent in bacterial isolates. **Aims:** This study was carried out to isolate the different microbial agents and evaluate their antibiograms pattern. **Methods:** Hospital based descriptive cross sectional study was conducted over the period of October 2021 to January 2022 at Nepalgunj Medical College, Kohalpur, Nepal. 292 urine samples were inoculated on cysteine lactose electrolyte deficient agar and incubated at 37 °C in incubator. After overnight growth, Gram positive and Gram negative bacteria were identified on the basis of different biochemical tests. Antibiotic sensitivity test was performed by Kirby-Bauer disk diffusion method. **Results:** Out of 292 urine samples, 28.76 % showed microbial growth. Majority of isolates were *Escherichia coli* and *Staphylococcus aureus*. Most vulnerable age group was 26-50 years. Overall pathogens were sensitive to amikacin, gentamicin, nitrofurantoin but resistant to cefixime. **Conclusion:** Regular surveillance of the resistance is required among microorganisms to ensure the appropriate therapy of Urinary tract infection.

**Keywords:** Antibiotic sensitivity test, Gram negative bacilli, Gram positive cocci, Resistance, Urinary tract infection

## Authors:

1. Dr. Kishor Gurung
2. Dr. Rupak KC
3. Dr. Ishant Pattel
4. Mr. Dhan Raj Neupane
5. Mr. Sandeep Pokhrel
6. Mrs. Samjhana Gurung

<sup>1</sup>Department of Microbiology and Infectious disease, Nepalgunj Medical College and Teaching Hospital, Nepal

<sup>2</sup>Intern Doctors, Nepalgunj Medical College, Nepal

<sup>3</sup>RHN Gurkha Welfare Trust, AWC, Dharan, Nepal

## Address for Correspondence:

Dr. Kishor Gurung  
Assistant professor  
Department of Microbiology  
Nepalgunj Medical College and Teaching Hospital, Kohalpur, Banke  
Email: kgurung95@gmail.com

## INTRODUCTION

Urinary tract infection (UTI) is defined as a disease caused by microbial invasion of the genitourinary tract that extends from the renal; cortex of kidney to the urethral meatus. Global incidence of UTI in 2019 was 404 million and mortality because of UTI increased by 140.18% as compared to 1990.<sup>2</sup> Females are at greater risk of developing a UTI than male. Occurrence of UTI is very common in female due to shorter urethra, large bacterial load in uroepithelial mucosa, obstruction in the urinary tract, sexual activity and pregnancy. The Common symptoms are frequent urination, discolored urine, pain when urinating and pelvic or rectal pain.<sup>3</sup> The prevalence of UTIs increases with age.<sup>4</sup> The prevalence of UTI rates across the Nepal show in varies, 21.3 %<sup>5</sup>, 27.1%<sup>6</sup>, 23.34%<sup>7</sup>, 37.84%<sup>8</sup>. The commonest bacterium of urinary tract infection is *Escherichia coli* (*E. coli*)<sup>6</sup> with isolation rates between 55-90 percent.<sup>3</sup> Other possible pathogens are *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp.,<sup>6</sup> *E. faecalis*,

*Citrobacter freundii*, *Enterobacter aerogenes* and Coagulase Negative Staphylococci (CoNS). In recent years, widespread use of antibiotics has resulted in an increasing incidence of antibiotic resistance among the urinary tract pathogens all over the world. Worldwide, emerging of antibiotic resistance is increasing among the urinary pathogens.<sup>9</sup> Antibiotics are the typical treatment for a UTI and the drugs most commonly recommended are sulfamethoxazole-trimethoprim, amoxicillin, nitrofurantoin, ampicillin, ciprofloxacin and levofloxacin.<sup>3</sup>

## METHODS

This study was hospital based descriptive cross sectional study. This study was conducted over a period of October 2021 to January 2022 at Nepalgunj Medical College, Kohalpur, Nepal. The sample size was calculated and was 292.

## Sample Collection

The mid-stream urine (10–20ml) samples were collected in the sterile dry, wide-mouthed, leak-proof container. Patient's details were recorded along with the urine sample. The urine sample were transferred to the microbiology laboratory and processed within two hours. Samples were processed following the Clinical and Laboratory Standard Institute (CLSI) guidelines 2021.<sup>10</sup>

## Sample Processing and bacterial identification

A loopful of urine sample was streaked with the help of a nichrome wire loop of 4mm (0.004 ml) on cysteine lactose electrolyte deficient (CLED) agar media. The plates were incubated at 37°C in incubator for 24 hours. After overnight, growth was observed and Gram stain of colony was performed to identify it as Gram positive cocci (GPC) or Gram negative bacilli (GNB). If growth was not seen on media, again plates were kept for another 24 hours. Catalase test and slide coagulase test were performed in any GPC colony for presumptive identification of Staphylococcus. Oxidase test was performed in NLF (non-lactose fermenting) colony to rule out Pseudomonas. Indole test, triple sugar iron (TSI) test, Urease test and Citrate test were put in GNB growth. All biochemical tests were incubated in incubator at 37 °C for 24 hours. Colony count of growth was determined by multiplying 100 to number of colony as CFU/ml. Any count more than 105 CFU/ ml was considered significant. Inoculums of growth in peptone water was made and adjusted to 0.5 McFarland as per CLSI (clinical and laboratory standard institute) guidelines.<sup>11</sup> Then the inoculums were seeded into Mueller Hinton agar (MHA) by lawn culture method with the help of sterile swab stick. The antibiotics susceptibility testing was done in Mueller Hinton agar following CLSI guidelines 2021.<sup>12</sup>

## Antibiotic Panel

There were 14 antibiotics tested namely, amikacin (AK:30µg), ampicillin/sulbactam (A/S:10/10µg), amoxicillin/clavulanic acid (AMC:20/10µg), cefixime (CFM:5µm), cefpodoxime (CPD:10µg), ciprofloxacin (CIP:5µg), clindamycin (CD:2µg), cotrimoxazole (COT:25µg), doxycycline (DO:30µg), gentamicin (GEN:10µg), meropenem (MRP:10µg), nitrofurantoin (NIT:300 µg), norfloxacin (NX:10µg), ofloxacin (OF:5µg). Isolates were classified as sensitive, intermediate and resistant consistent with the standardized table supplied by quality control limits for antibiotics from HiMedia<sup>13</sup> and Biogram<sup>14</sup>.

## Data Analysis

Data collection was done in preformed data collection sheet and results. Collected data was coded as per variables and enter in Microsoft Office Excel Worksheet and analyzed. Categorical variables were described with counts.

## RESULTS

There were 292 urine samples examined in this study, 86 (29.45%) samples were collected from male among which 26 (8.90%) were found to be growth positive and 206 (70.55%)

samples were collected from female among which 58 (19.86%) were found to be growth positive. The hospital prevalence of UTI was 84 (28.76 %). The male to female ratio was 1:2.39. (Table I). Out of 116 patients, 91 were vaccinated and enrolled in the study.

Gender	Total	Isolates		
		Positive	Prevalence (%)	Negative
Male	86	26	30.23%	60
Female	206	58	28.15%	148
Total	292	84	28.76%	208

**Table I: Gender and isolates wise distribution of patients**

Among 84 growths, bacterial isolates were 81 (27.73%) and yeast cells in 3 (1.03%) samples. Out of 81 bacterial isolates, 34 were GPC and 47 were GNB. Three species of GPC were grown, Staphylococcus aureus (35.71%) was isolated as most prevalent and Enterococcus faecalis (1.19%) was the least frequent bacteria. Similarly, four species of GNB were isolated during the study. The most prevalent bacteria obtained were E. coli (32.14%) and Acinetobacter spp (2.38%) was the least frequent bacteria is presented in Table II

Bacteria	Number	Percentage (%)
Staphylococcus aureus	30	35.71
GPC Streptococcus spp	3	3.58
Enterococcus faecalis	1	1.19
E. coli	27	32.14
GNB Klebsiella spp	12	14.28
Pseudomonas spp	6	7.14
Acinetobacter spp	2	2.38
Yeast	3	3.58
Total	84	100

**Table II: Bacterial/Yeast cells isolated from urine samples**

Age-wise distribution of patients showed that the highest number of bacterial growth 54 (64.28%) falls in the age group of 26-50 years then followed by 18 (21.42%) in the age group less than 25 years, 11 (13.09%) in the age group of 51-75 years and 1 (1.19%) in the age group in more than 76 years. The association of UTI with different age group and gender of the patients (Table III).

Age	Male	Female	Isolate (%)
0-25	21	70	18 (6.17)
26-50	49	115	54 (18.49)
51-75	12	19	11 (3.76)
>76	4	2	1 (0.34)
Total	86	206	84 (28.76)

**Table III: Age and gender wise distribution in relation to isolates**

There were 14 antibiotics tested against different bacterial isolates in this study. The majority *Staphylococcus aureus* and *E. coli* were found to be resistant against cefixime but sensitive to doxycycline. *Pseudomonas* was sensitive to amikacin and gentamicin but resistant against almost all the tested antibiotics. Isolates and antimicrobial sensitivity are shown in Table IV.

Antibiotics/ Bacteria	GPC			GNB			
	S. aureus	Strept spp	E. faecalis	E. coli	Kleb spp	Pseudo	Acineto
AK	S	3 (10%)	-	-	7 (26%)	2 (17%)	2 (33%)
	I	-	-	-	-	-	-
	R	-	-	-	-	-	-
AS	S	-	-	-	-	-	-
	I	-	-	-	-	-	-
	R	2 (6%)	-	-	-	2 (17%)	1 (17%)
AMC	S	18 (60%)	1 (33%)	-	5 (19%)	6 (50%)	-
	I	6 (20%)	-	-	10 (37%)	3 (25%)	1 (50%)
	R	2 (6%)	-	1 (100%)	7 (26%)	2 (17%)	5 (83%)
CFM	S	3 (10%)	1 (33%)	-	7 (26%)	1 (8%)	-
	I	7 (23%)	1 (33%)	-	7 (26%)	3 (25%)	-
	R	17 (56%)	-	-	13 (48%)	3 (25%)	4 (67%)
CPD	S	6 (20%)	2 (66%)	-	6 (22%)	3 (25%)	-
	I	5 (16%)	-	-	4 (15%)	3 (25%)	1 (50%)
	R	-	-	-	-	-	2 (33%)
CIP	S	8 (26%)	2 (66%)	-	5 (19%)	3 (25%)	3 (50%)
	I	8 (26%)	-	-	5 (19%)	1 (8%)	2 (100%)
	R	2 (6%)	-	-	6 (22%)	1 (8%)	-
CD	S	5 (16%)	-	-	-	-	-
	I	1 (3%)	-	-	-	-	-
	R	-	-	-	-	-	-
COT	S	6 (20%)	1 (33%)	-	7 (26%)	3 (25%)	1 (50%)
	I	6 (20%)	-	-	1 (3%)	3 (25%)	1 (50%)
	R	8 (26%)	-	1 (100%)	9 (33%)	1 (8%)	3 (50%)
DO	S	15 (50%)	1 (33%)	-	13 (48%)	3 (25%)	2 (100%)
	I	2 (6%)	1 (33%)	1 (100%)	4 (15%)	2 (17%)	2 (33%)
	R	8 (26%)	-	-	3 (11%)	3 (25%)	3 (50%)

GEN	S	8 (26%)	2 (66%)	1 (100%)	-	4 (33%)	1 (17%)	1 (50%)
	I	-	-	6 (22%)	-	-	-	-
	R	-	-	-	2 (7%)	-	-	-
MRP	S	1 (3%)	-	-	1 (3%)	2 (17%)	1 (17%)	-
	I	-	-	-	-	-	-	-
	R	-	-	-	-	-	-	-
NIT	S	17 (60%)	2 (66%)	1 (100%)	17 (63%)	4 (33%)	-	2 (100%)
	I	6 (20%)	-	-	2 (7%)	5 (42%)	-	-
	R	2 (6%)	-	-	1 (3%)	-	5 (83%)	-
NX	S	-	1 (3%)	-	1 (3%)	1 (8%)	-	-
	I	5 (16%)	1 (3%)	-	-	1 (8%)	1 (17%)	-
	R	-	-	-	3 (11%)	-	1 (17%)	-
OF	S	2 (6%)	-	-	4 (15%)	2 (17%)	-	-
	I	6 (20%)	-	-	1 (3%)	1 (8%)	-	-
	R	1 (3%)	1 (33%)	-	6 (22%)	3 (25%)	2 (33%)	-
<b>Total</b>		<b>30</b>	<b>3</b>	<b>1</b>	<b>27</b>	<b>12</b>	<b>6</b>	<b>2</b>

Table IV: Number of antimicrobial sensitivity against different bacterial isolates

*Staphylococcus aureus* (*S. aureus*), *Streptococcus* spp. (*Strept*), *Enterococcus faecalis* (*E. faecalis*), *Echerichia coli* (*E.coli*), *Klebsiella* spp. (*Kleb. spp.*), *Pseudomonas* spp (*Pseudo*), *Acinetobacter* spp (*Acineto*)

## DISCUSSION

The study was done to isolate the different microorganism and evaluate the current antimicrobial susceptibility pattern. Numbers of females are higher than male. The hospital prevalence of UTI was 28.76 % which was higher in comparison to the finding of 19.7%<sup>15</sup> but lower in comparison of 37.4%.<sup>16</sup> There are many methods used to calibrate the analysis of bacterial uropathogens.

Among 292 bacterial isolates, 59.52% were GNB and 40.48% were GPC. In this study, GNB were slightly more encountered than GPC. There are three types of GPC and four types of GNB isolated in this current study. Among them, *Staphylococcus aureus* (35.71%) is was commonest in GPC followed by *Streptococcus* spp (3.58%) and *Enterococcus faecalis* (1.19%). Similarly, *E. coli* (32.14%) encountered as higher percentage in GNB followed by *Klebsiella* spp (14.28%), *Pseudomonas* spp (7.14%) and *Acinetobacter* spp (3.58%). This finding was similar with study done at Bharatpur by Adhikari S<sup>6</sup> and in Kathmandu by Raza S.<sup>15</sup>

In this study, the urinary isolates showed high degree of resistance to cefixime but sensitive to amikacin, nitrofurantoin,

ciprofloxacin and gentamicin. The above mentioned result correlates with study conducted by S. Raza.<sup>15</sup>

## LIMITATIONS

There are different environmental conditions, different factors like sexual behavior (sexually active individuals and unnatural sexual activities are more prone to exposed the UTI), lack of sanitary materials such as access of water, host factors, hygiene practices, different socioeconomic status and also not studied the underlying primary disease which predispose to UTI like urinary tract stone, diabetes mellitus, immunocompromised patients. These confounding factors are not studied which might have influenced the result. So the results of this study cannot be generalized.

## CONCLUSION

In GNB, *Klebsiella* spp. was the second most common isolates after *E.coli* in this study. Isolates were most resistant to cefexime, ampicillin and were most sensitive to nitrofurantoin and meropenem, amikacin, ciprofloxacin and gentamicin. Clindamycin and amoxicillin/clavulanic acid were still useful for better result against GPC.

## REFERENCES

- Baveja CP. Textbook of microbiology. New Delhi: Arya publications. 3rd edition. 2011
- Zhu C, Wang DQ, Zi H, Huang Q, Gu JM, Li LY, Guo XP, Li F, Fang C, Li XD, Zeng XT. Epidemiological trends of urinary tract infections, urolithiasis and benign prostatic hyperplasia in 203 countries and territories from 1990 to 2019. *Mil Med Res*. 2021 Dec 9;8(1):64. doi: 10.1186/s40779-021-00359-8. PMID: 34879880; PMCID: PMC8656041.
- Basnyat B. Situation analysis and recommendations. Antibiotic use and resistance in Nepal. [Internet]. 2022 [cited in 2 February 2022]. Available from <https://www.nphfoundation.org/wp-content/uploads/2017/05/6904671627th-july-final-file-GRAP.pdf>
- Schmiemann G, Kniehl E, Gebhardt K. The diagnosis of urinary tract infection: a systematic review. *Dtsch Arztebl Int*. 2010; 107: 361–67
- Gautam G, Regmi S, Magar NT, Subedi B, Sharma T, Regmi SM. Occurrence of urinary tract infection among children attending Gandaki Medical College Teaching Hospital and Research Center, Pokhara, Nepal. *Int J Infect Microbiol*. 2013;2(3):82-6 DOI: <http://dx.doi.org/10.3126/ijim.v2i3.8665>
- Adhikari, S., Khadka, S., Sapkota, S., Rana, J. C., Khanal, S., Neupane, A., & Sharma, B. (2019). Prevalence and Antibigrams of Uropathogens from the Suspected Cases of Urinary Tract Infections in Bharatpur Hospital, Nepal. *JCMS*. 2019;15(4):260–66. <https://doi.org/10.3126/jcmsn.v15i4.20856>
- Baral R, Timilsina S, Jha P, Bhattarai NR, Poudyal N, Gurung R, Khanal B, Bhattacharya SK. Study of antimicrobial susceptibility pattern of Gram positive organisms causing UTI in a tertiary care hospital in eastern region of Nepal. *Health Renaissance*. 2013;11 (2):119-24.
- Thakur S, Nagpal KL. Urinary Tract Infection in Pregnant Women at Kathmandu, Nepal. *JMCRR*. 2020; 3(9): 454–58. DOI: <https://doi.org/10.15520/mcrr.v3i9.143>
- Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO-sens project. *J Antimicrob Chemother*. 2003;51:69-76.
- UF health. [Internet] 2022 [cited in 2 February 2022] Available in <https://pathlabs.ufl.edu/client-services/specimen-shipping/specimen-collection-procedures-urology/>
- Baron EJ, Tenover FC, Tenover MC. Microorganisms encountered in the urinary tract. In: Bailey & Scott's diagnostic microbiology. 9th edition. St. Louis, Missouri: Mosby publishers; 1994.
- Performance standards of antimicrobial susceptibility testing; Ninth edition Vol. 26. No. 1 Clinical Laboratory Standards Institute; 2006.
- HiMedia. [Internet] 2022 [cited in 2 February 2022] Available in <https://www.himedialabs.com/HML/images/literature/pdf/100000027/68.pdf>
- Biogram. [Internet] 2022 [cited in 2 February 2022] Available in <http://www.tulipgroup.com/MicroExpress/Biogram%20%20Packinsert%202015-16.pdf>
- Raza S, Pandey S, Bhatt CP. Microbiological Analysis of the Urine Isolates in Kathmandu Medical College Teaching Hospital, Kathmandu, Nepal. *Kathmandu Univ Med J* 2011;36(4):295-7.
- Rai SK, Pokhrel BM, Sharma AP. A prospective study of antibiotic sensitivity profile of the organisms associated with clinical infections among the patients attending TU Teaching Hospital. *J Nepal Assoc Med Lab Sci* 2001; 3:13-6.