



**ORIGINAL RESEARCH PAPER**

**Microbiology**

**DETECTION OF COMMON URINARY PATHOGENS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERNS AMONG SUSPECTED CASES OF URINARY TRACT INFECTION IN A TERTIARY CARE HOSPITAL OF RUPANDEHI DISTRICT OF NEPAL**

**KEY WORDS:** Urinary tract infection, MDR strain, Amikacin

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**ABSTRACT**

**Introduction :** UTI is the most common bacterial infection, accounting for 25% of all infections. UTI can occur in any populations and age groups however, infection is most common in women in reproductive age.

**Objectives :** The present study was conducted to determine the common causative agents of UTI at a tertiary care hospital among outpatient and inpatient department and their antibiotic susceptibility pattern.

**Materials and methods :** A prospective observational study was conducted at the Department of Microbiology of Devdaha medical College and Research institute. Fresh mid stream urine sample was collected from the patients who were suspicious of having urinary tract infection. Clinical isolates were obtained using conventional method of bacterial culture. Isolated bacteria were subjected to assess antimicrobial susceptibility testing by Modified Kirby Bauer disc diffusion technique.

**Results :** Out of 414 urine samples, 140 (33.8%) revealed significant growth. Incidence of urinary tract infections was higher in females than males. *Escherichia coli* (48.6%) was found to be the most predominant agent followed by *Klebsiella* species (31.4%). Amikacin (80.2%) was the most effective antibiotic followed by Nitrofurantoin (79.9%). Ampicillin was found to be least effective antibiotic with sensitivity percentage of (18.9%).

**Conclusion :** The present study shows majority of pathogens causing UTI is developing resistance to multiple groups of antibiotics. On the basis of our study, we recommend Amikacin and Nitrofurantoin as best drug of choice to treat UTI. Along with the proper selection of antibiotic we should limit the extensive use of antibiotic to reduce the occurrence of MDR strains.

**Introduction**

Urinary tract infections (UTI) could be defined as the persistent presence of actively multiplying microorganism within the urinary tract. UTI implies both microbial colonization of the urine and invasion of the lower or upper urinary tract by microorganisms.<sup>(1)</sup> UTI is the most common bacterial infection, accounting for 25% of all infections. UTI can occur in any populations and age groups however, infection is most common in women in reproductive age.<sup>(2,3)</sup> The increase risk factor for UTI in women may be due to short urethra, absence of prostatic secretions, pregnancy and easy contamination of urinary tract with faecal flora.<sup>(4)</sup>

Urinary tract infection (UTI) is one of the most important cause of morbidity in the general population, and is the second most common cause of hospital visits.<sup>(5)</sup> Gram negative enteric bacilli, especially *Escherichia coli* and *klebsiella spp* are the leading pathogens though *Enterococcus spp*, yeasts and *Staphylococcus aureus* have emerged as prominent agents of UTI in recent years.<sup>(6)</sup> Antimicrobial therapy is the core treatment for UTIs, with the main objective being the eradication of bacterial growth in the urinary tract through an efficacious, safe and cost-effective antimicrobial agents.<sup>(7)</sup> Clinical experience has indicated the presence of numerous cases of antibiotic resistance to common antibiotics by uropathogens in both developed and developing countries.<sup>(8)</sup> This may be due to the extensive uses of antimicrobial agents which in recent years, has become a major problem worldwide.<sup>(9)</sup>

In the present context of Nepal, we need an appropriate antimicrobial therapy to reduce the possible complication of UTI. Such type of data regarding the susceptibility pattern of urinary pathogens has not been published in Rupandehi district of Nepal till date. Hence, the present study was carried out to determine the common causative agents of UTI at a tertiary care hospital among outpatient and inpatient department and their antibiotic susceptibility pattern. It will be helpful for the clinicians in the empirical treatment and care of the patient having UTI because the frequencies of isolated pathogens and their antimicrobial resistance patterns can vary in different geographical regions.<sup>(10)</sup>

**Materials and Methods:**

A prospective observational study was conducted at the Department of Microbiology of Devdaha medical College and Research institute.

**Study period :**

The study period was from January 2015 to August 2017.

**Study population :**

A total of 414 urine specimens were collected from the patients attending IPD and OPD of various clinical services with a suspected case of urinary tract infection.

**Specimen collections and storage**

Freshly voided mid stream urine sample were collected in a sterile wide mouth container. All the specimens were processed within one hour after the collection for aerobic bacterial culture. If delayed specimens were refrigerated and processed within 4-6 hours

**Processing of the specimens :**

Semiquantitative technique was applied to perform urine culture. A loopful of well mixed uncentrifuged urine was inoculated on the surface of blood agar and MacConkey agar media. All plates were then incubated at 37° C aerobically for 24 hours.

**Identification of isolates:**

Isolates were identified by colony morphology and Gram staining. Gram negative bacteria were further identified on the basis of motility test and various biochemical reactions such as sulphide indole test medium, methyl red medium, citrate utilization test, urease production and triple sugar iron medium. Gram positive bacteria was identified on the basis of catalase production and coagulase test.<sup>(11)</sup> Patients who had significant bacteriuria (>10<sup>5</sup>colony forming unit/ml urine) were only included in the microbiological study.

**Antimicrobial susceptibility test:**

Isolates were subjected to antimicrobial susceptibility testing by using Kirby Bauer disc diffusion method as per CLSI guidelines. The following antibiotic disc (Hi Media Laboratory, Mumbai, India) were used :norfloxacin (10 µg), levofloxacin (5 µg), nitrofurantoin (300 µg), ampicillin (10 µg), amikacin (30 µg), cefixime ( 5 µg), chloramphenicol (10 µg), ceftriaxone (30 µg), gentamicin(10 µg) and cotrimoxazole(25 µg).Results were interpreted as sensitive or resistant and compared with standard chart as for standard strain. The ATCC standard strain of *Escherichia coli* (25922), *Staphylococcus aureus* (25923) and *Pseudomonas aeruginosa* (27853) were used as quality control throughout the study for culture and antimicrobial susceptibility test.<sup>(12)</sup>

**Identification of Multidrug resistant (MDR) strains:**

MDR isolates were identified according to the guidelines recommended by international expert committee of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC).<sup>(13)</sup> In this study, the isolates resistant to at least one antibiotic from three different group of first line drugs tested was determined as MDR.

**Inclusion criteria :**

Specimens of patients with or without signs and symptoms of UTI with no age barrier were included in the study.

**Exclusion criteria :**

Unsterile specimens, time delayed specimens for culture (after one hours of collection) which were not kept refrigerated at 4°C and inadequate specimen for urine culture were excluded from the study.

**Statistical analysis**

Data were analysed using SPSS version 16 for windows. Statistical test of significance and probabilities was calculated by applying Chi-square test. A p-value of less than or equal to 0.05 was considered to be statistically significant (p≤0.05).

**Results:**

A total of 414 urine specimens were processed in our laboratory, among which 140(33.8%) specimens revealed significant growth.

**Table 1: Gender distribution of culture positive isolates**

Gender	Frequency	Percentage
Female	119	85
Male	21	15
Total	140	100

Among 140 isolates of suspected cases of urinary tract infection, females (85%) were found to be highly infected as compared to males (15%)

**Table 2: Age distribution of culture positive cases of UTI**

Age group	Frequency	Percentage
Below 1 month	0	0
1 months-1 year	1	0.7
2-5 year	4	2.9
6-15 year	4	2.9
16-45 year	91	65
46-60 year	18	12.8
Above 60 year	22	15.7
Total	140	100

**Table 6: Sensitivity pattern of different organisms to different antibiotics**

Antibiotics	NX	NIT	AMP	GEN	AK	CTR	LE	C	AZM	COT	CFM
Samples	115	139	106	89	116	97	106	86	17	98	125
organisms	n %	n %	n %	n %	n %	n %	n %	n %	n %	n %	n %
E.coli	22(19.1)	57(41)	11(10.3)	7(7.8)	50(43.1)	20(20.6)	24(22.6)	34(39.5)	0	21(21.4)	15(12)
Kleb	17(14.7)	32(23)	6(5.7)	11(12.3)	38(32.8)	12(12.4)	10(9.4)	15(17.4)	0	9(9.2)	12(9.6)
Staph	11(9.5)	20(14.3)	2(1.9)	13(14.6)	0	3(3.1)	11(10.4)	14(16.3)	9(53)	7(7.1)	3(2.4)
Pseudo	2(1.7)	2(1.4)	1(0.9)	2(2.2)	4(3.4)	0	3(2.8)	1(1.2)	0	2(2)	1(0.8)
Proteus	1(0.9)	0	0	0	1(0.9)	0	1(0.9)	1(1.2)	0	0	1(0.8)
Total	53(46.1)	111(79.9)	20(18.9)	33(37.1)	93(80.2)	35(36.1)	49(46.2)	65(75.6)	9(52.9)	39(39.8)	32(25.6)

N: Number of samples, NX: Norfloxacin, NIT: Nitrofurantoin, AMP: Ampicillin, GEN: Gentamicin, AK: Amikacin, CTR: Ceftriaxone, LE: Levofloxacin, C: Chloramphenicol, AZM: Azithromycin, COT: Cotrimoxazole, CFM: Cefixime, E.coli: *Escherichia coli*, Kleb: *Klebsiella species*, Staph: *Staphylococcus aureus*, Pseudo: *Pseudomonas species*, Proteus: *Proteus species*

Among all isolated bacteria, higher percentage (80.2%) of susceptibility was observed for Amikacin followed by Nitrofurantoin (79.9%) and least for Ampicillin (18.9%) as documented in Table 6.

Among the isolates, the highest percentage (65%) of culture positivity was found within the age group of 16-45 years, however the lowest percentage of the patient were with the age group of one month to one year. Patients having the age below one month were not infected with any bacterial agents as shown in Table 2

**Table 3: Bacteria isolated from indoor and outdoor patients**

Department	Frequency	Percentage	P value
OPD	118	84.3	0.183
IPD	22	15.7	
Total	140	100	

Among 140 isolates, a higher percentage (84.3%) of infection were found in outdoor patients as compared to indoor patients (15.7%), as observed in Table 3

**Table 4: Pattern of bacterial isolates of UTI suspected cases**

Bacteria	Frequency	Percentage
<i>Escherichia coli</i>	68	48.6
<i>Klebsiella species</i>	44	31.4
<i>Staphylococcus aureus</i>	22	15.7
<i>Pseudomonas species</i>	5	3.6
<i>Proteus species</i>	1	0.7
Total	140	100

Total five species of bacteria were isolates among which, *Escherichia coli* (48.6%) was found to be the most predominant followed by *Klebsiella species* (31.4%), *Staphylococcus aureus* (15.7%), *Pseudomonas species* (3.6%) and *Proteus species* (0.7%) as given in Table 4

**Table 5: Antibiotic sensitivity pattern of different antibiotics**

Antibiotics	No.of samples	Sensitive (%)	Resistant (%)
Norfloxacin	115	53(46.1)	62(53.9)
Nitrofurantoin	139	111(79.9)	28(20.1)
Ampicillin	106	20(18.9)	86(81.1)
Gentamicin	89	33(37.1)	56(62.9)
Amikacin	116	93(80.2)	23(19.8)
Ceftriaxone	97	35(36.1)	62(63.9)
Levofloxacin	106	49(46.2)	57(53.8)
Chloramphenicol	86	65(75.6)	21(24.4)
Azithromycin	17	9(52.9)	8(47.1)
Cotrimoxazole	98	39(39.8)	59(60.2)
Cefixime	125	32(25.6)	93(74.4)

Antimicrobial susceptibility pattern of isolated organism revealed that Amikacin (80.2%) was the most effective antibiotic followed by Nitrofurantoin (79.9%). Ampicillin was found to be least effective antibiotic with sensitivity percentage of (18.9%).

**Table 7: Distribution of MDR uropathogens**

Organism	Frequency	Percentage	Total
<i>Escherichia coli</i>	53	77.9	68
<i>Klebsiella species</i>	26	59.1	44
<i>Staphylococcus aureus</i>	13	59.1	22
<i>Pseudomonas species</i>	4	80	5
<i>Proteus species</i>	1	100	1
MDR	97	69.3	140

Out of 140 isolates, 97 (69.3%) were found to be multi drug resistant of which the highest percentage of MDR was found in

strain of *Proteus species* (100%) followed by *Pseudomonas species* (980%) and *Escherichia coli* (77.9%).

**Discussion**

In the present study, out of 414 sampled processed, 140(33.8%) were found to be culture positive which is in agreement with the study done by Baral P et al<sup>(8)</sup> (30.8%) and Singh RK et al<sup>(14)</sup>(34%).

In our study, the occurrence of urinary tract infection is higher in females (85%) than in males (15%). This is in accordance with the study reported by Singh RK et al<sup>(14)</sup> where the percentage of organisms causing UTI was 83.59% from women and 16.1% from men. Other studies are also correlated with these data (Das RN et al, Moue A et al and Aryal B et al).<sup>(5,9,15)</sup> Mainly women are more prone to get UTIs than men because of short urethra and closer to anus.

In the present study, the highest percentage (65%) of culture positivity was found within the age group of 16-45 year which is similar to the study done by Singh RK et al<sup>(14)</sup>(20-49 years), Khadka KS et al<sup>(16)</sup> (21-40 years)

In the present study, *Escherichia coli* (48.6%) was found to be the most predominant cause of UTI followed by *Klebsiella species* (31.4%). This is in accordance with the study done by<sup>1</sup> G MN et al,<sup>(4)</sup>Das RN et al,<sup>(5)</sup>Kaur N et al,<sup>(6)</sup>Sujatha R et al<sup>(10)</sup> Aryal B,<sup>(15)</sup> and Gupta Set al.<sup>(17)</sup>

Our study revealed that Amikacin (80.2%) was the most effective antibiotic followed by Nitrofurantoin (79.9%). Similarly Ampicillin was found to be the least effective antibiotic with sensitivity percentage of (18.9%). This is comparable with the documentation of Thapa P e t al<sup>(2)</sup> whereas the study done by Sujatha R et al<sup>(10)</sup> expressed that ampicillin was the most sensitive antibiotic with 61% sensitivity. The antimicrobial sensitivity and resistance pattern vary from community to community and from hospital to hospital. This is because of emergence of resistant strains, caused by indiscriminate use of antibiotics.

In our study, the percentage of MDR strain was 69.3% which is similar to the study of Parajuli NP et al<sup>(18)</sup> (64.9%).

**Conclusion**

Urinary tract infection is the most common problem seen in developing countries like Nepal. We observe *Escherichia coli* as the predominant organism causing UTI. UTI can be community acquired as well as hospital acquired. The present study shows majority of pathogens causing UTI is developing resistance to multiple groups of antibiotics. The routinely applied antibiotics are generating these types of issues which is directly affecting in the empirical treatment and cure of UTI. On the basis of our study, we recommend Amikacin and Nitrofurantoin as the best drug of choice to treat UTI. Along with the proper selection of antibiotic we should limit the extensive use of antibiotic to reduce the occurrence of MDR strains. To overcome all these problems, routine surveillance of antibiotic susceptibility testing should also be followed by the physicians and laboratory personals.

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