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### Original Article

## Incidence and Susceptibility of Uropathogens Isolated among the Patients at Tertiary Care Hospital in Eastern Nepal.

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### Abstract:

#### Background

Urinary Tract Infection (UTI) is one of the most common infectious diseases which affect almost all ages groups of population. Production of  $\beta$ -lactamases is responsible for antibacterial resistance which is frequently observed in *Enterobacteriaceae* isolates, particularly by *E. coli* and *Klebsiella pneumoniae*. This investigation has been carried out to determine the current status of prevalence and susceptibility of uropathogens isolated among the patients at tertiary care hospital in eastern Nepal.

#### Material and Methods

This study was done at the department of Microbiology, Nobel Medical College Teaching Hospital, Biratnagar, Nepal during May 1<sup>st</sup> 2015 to October 31<sup>st</sup> 2015. Midstream clean-catch urine was sampled from 1730 suspected urinary tract infection patients of different age and sex groups. Uropathogens were recognized in term of standard and specific microbiological techniques and antimicrobial susceptibility pattern was determined by Kirby Bauer Disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines.

#### Results

Out of 1730 suspected specimens Culture resulted a total of 761 (43.98 %) positive and 969 (56.02%) negative among that significant growths of uropathogens including 700 (91.98 %) unimicrobial and 60 (7.88 %) polymicrobial growths. In term of Gender distribution 443 (25.60 %) were male and 1287 (74.40 %) were female hence the ratio is 0.34:1, respectively. *E. coli* was the leading isolate (66 %), followed by *Klebsiella spp.* (12 %), *Enterococcus spp.* (8 %), *Pseudomonas spp.* (6 %), *Acinetobacter anitratus* (5 %), *Proteus spp.* (3 %).

#### Conclusion

The high frequency of multidrug resistance in bacterial uropathogens was seen. Principally, resistance patterns were seen higher for amoxycillin, co-trimoxazole, flouroquinolones and third-generation cephalosporins, Existing uropathogens highlights the highest rate of vulnerability to nitrofurantoin, amikacin and gentamicin which provide much better antibiotic coverage and can be adapted for practical treatment of urinary tract infections.

**Key words:** *Antimicrobial susceptibility, mid-stream urine (MSU) , uropathogens, Urinary tract infection(UTI)*

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#### Introduction

Urinary tract infection(UTI) is the most common in Nepal and most common

nosocomial infection [1]. In urinary tract infections (UTIs) cystitis and pyelonephritis are considered to be the 2<sup>nd</sup> most common

infections, they account for about 150-250 million cases globally per annum [2,3]. Due to rational uses of antibiotic and other factors, the resistance of antibiotic is increasing and thus it is necessary to monitor the resistance pattern for getting better empirical therapy [4]. Most of the Uropathogens have made resistance to normally used drugs, which narrows the area of an effective treatment. -lactamases enzymes production is the most common resistance mechanisms [5]. The specimen shows  $> 10^5$  organisms per ml which refers to considerable bacteriuria and mainly caused by, *Escherichia coli* which is responsible for  $> 75\%$  of cases [6, 7]. Other leading agents are *Enterobacteriaceae*, *Staphylococcus saprophyticus* and *Enterococcus faecalis*. Most commonly UTI are caused by unimicrobial but polymicrobial infections also take place [8].

#### **Materials and Methods**

The study was undertaken with the uropathogens isolates obtained from specimens of patients visited for a period of 6 month from May 1<sup>st</sup> 2015 to October 31<sup>st</sup> 2015, in the department of Microbiology in a tertiary care hospital Nobel Medical College and Teaching Hospital, Biratnagar, Nepal. The study was deputed after approval of ethical committee and approved by Institutional Review Committee (IRC).

Selection of cases & inclusion criteria: Written consents were taken from all cases prior to the inclusion in the study. All suspected patients of UTI were interviewed directly with prearranged questionnaire to collect data about type of patient (hospitalized- or out-patient), symptoms, prior history of UTI, and underlying diseases. Only those cases shown at least one of the clinical features of UTI (dysuria, frequency, or prior history of UTI) were included. A midstream and/or catheter-catch urine sample is included. Microscopic

demonstration of pus cells  $> 5$ /HPF (high power field) in a centrifuged deposit of urine was included. Patients who did not have a course of antibiotics at least two weeks prior.

Exclusion Criteria:

1. Those clinical samples which shows polymicrobial & insignificant growth.
2. Patients with a course of antibiotics at least two weeks' prior.
4. Incomplete culture form, without proper labelling (date, time, age, lab number and sex), were excluded.

The study population included patients visiting the hospital suspected of UTIs. Patients included in the study were given pre-labelled leak proof, sterile, screw-capped container to collect the mid-stream urine (MSU) sample. Urine samples from all age group were included in the study. The collected urine specimens were processed in the Microbiology laboratory within 2 hr of collection. Microscopic demonstration of pus cells  $> 5$ /HPF (high power field) in a centrifuged deposit of urine sample were streaked directly on MacConkey agar (MA) plates and Blood agar (BA) plates. These plates were incubated at  $37^\circ\text{C}$  aerobically and after overnight incubation, they were checked for bacterial growth. The isolates were identified by their colony morphology, Gram staining, catalase test, oxidase test, and other appropriate biochemical tests as per standard laboratory methods of identification. Antibiotic susceptibility testing of bacterial isolates was done by Kirby Bauer disk diffusion method following CLSI guidelines using Mueller Hilton Agar (MHA) [9]. The antibiotic discs were obtained from HiMedia Laboratories (India). An isolate was considered as MDR if it was resistant to three or more drugs of different classes/groups of antibiotics as for CLSI guidelines.

#### **Results**

A total of 1730 cases of different age and sex those who fulfilled the inclusion criteria

of suspected UTI were included in this study. Of 1730 cases, 443 (25.60 %) were male and 1287 (74.40 %) were female. Culture of 1730 urine samples yielded a total of 761 (43.98 %) positive bacterial growths and 969 (56.02%) negative, including 700 (91.98 %) unimicrobial (single bacterial species) and 60 (7.88 %) polymicrobial (pair of two different bacterial species) growths. *E. coli* was the predominant isolates 503 (66 %), followed by *Klebsiella spp.* 91 (12 %), *Enterococcus spp.* 61 (8 %), *Pseudomonas spp.* 45 (6 %), *Acinetobacter anitratus* 38 (5 %), *Proteus spp.* 23 (3 %). Out of 503 (66%) *E.coli*, MDR *E.Coli* was 54 (10.73%) which shown sensitive to Nitrofurantoin 88%, Amikacin 65%, Imipenem 65%, Azetronam 53%, Ampicillin-Salbactam 35%.

Culture positive & negative % is shown in table 1, gender distribution in table 2, Pattern of bacterial isolates in table 3. Antibiotic sensitivity of *E.coli*, *Klebsiella* & *Proteus* are shown in table 4. Table 5 shows sensitivity pattern of *Enterococcus* & Table 6 shows sensitivity pattern of *Pseudomonas*, *Acinetobacter* & MDR *E.coli*.

Table 1: Urine culture result

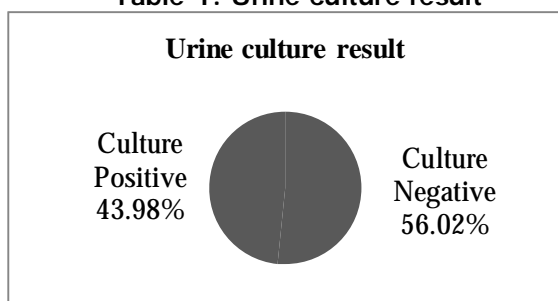


Table 2: Gender distribution for rate of isolation in urine culture

Males No ( % )	Females No ( % )
443 (25.60%)	1287 (74.40 %)

Table 3: Pattern of bacteria isolated from urine culture

Bacterial spp.	NO of isolates (%)	No of isolates
E.coli	66 %	503
Klebsiella spp.	12 %	91
Enterococcus spp.	8 %	61
Pseudomonas spp	6 %	45
Acinetobacter anitratus	5 %	38
Proteus spp	3 %	23
Total	100%	761

Table 4: Antibiotic Sensitivity Pattern Of E.Coli, Klebsiella spp. & Proteus.

Antibiotics sensitivity %	Isolates		
	E.coli	Klebsiella spp.	Proteus
NIT	94	70	80
GEN	93	70	40
CAZ	39	44	60
NX	41	63	60
AMP	5	4	20
CTX	39	44	60
CIP	8	10	NT

NIT;Nitrofurantoin , GEN;Gentamicin, CAZ;Ceftazidime, NX;Norfloxacin, AMP;Ampicillin, CTX;Cefotaxime, CIP;Ciprofloxacin, NT;Not tested.

Table 5: Antibiotic Sensitivity Pattern of Enterococcus spp.

Antibiotics	sensitivity %
NIT	79
NX	53
CIP	21
p	26
HLG	68
VA	95
TEI	89

NIT;Nitrofurantoin, NX;Norfloxacin, CIP;Ciprofloxacin, P;Penicillin, HLG;High Level Gentamycin, VA;Vancomycine, TEI;Teicoplanin.

**Table 6: Antibiotic Sensitivity Pattern Of Pseudomonas, Acinetobacter anitratus And MDR E.Coli.**

Antibioti CS sensitiv y %	Isolates		
	Pseudomon as spp.	Acinetobac ter anitratus	MD R E.Co li
NIT	10	20	88
GEN	NT	0	NT
CAZ	86	NT	0
NX	67	NT	0
AMP	13	NT	0
CTX	38	0	0
CIP	82	0	0
TEI	NT	NT	65
AK	91	80	65
IPM	100	60	65
PIT	20	0	6
COT	83	NT	12
AT	92	40	53
A/S	75	NT	35
LE	88	0	18
TOB	83	0	NT
CPM	29	NT	NT

NIT;Nitrofurantoin , GEN;Gentamicin,  
CAZ:Ceftazidime, NX;Norfloxacin, AMP;Ampicillin,  
CTX:Cefotaxime, CIP;Ciprofloxacin, TEI;Telcoplanin ,  
AK;Amikacin IPM;Imipenem, COT;Cotrimoxazole,  
AT;Azetronam,  
A/S; Ampicillin-Salbactam, LE;Levofloxacin,  
TOB;Tobramycin, CPM;Cefepime , NT;Not tested

### Discussion

Our research informs about the distribution and the antibiotic sensitivity pattern of uropathogens which is isolated from the UTI patients. Our research focussed that *Enterobacteriaceae* are most common contributory organism for UTI, a finding which is similar to the report published before [10]. In term of Gram-negative bacteria, highest rate of resistance has been seen towards first-line drugs like amoxicillin (89.75%), co-trimoxazole (53.6%), and norfloxacin (42.25%). Nonetheless, other drugs like amikacin and nitrofurantoin demonstrated the least resistance. Our research, which resembles

the previous reports, shows that *E. coli* is the leading contributing organism of UTIs with high rate of antibiotic resistance. In *E. coli* Cefotaxime, ceftazidime amoxicillin, ciprofloxacin, norfloxacin are proved to have very high frequency ranging from 61% to 95 % of resistance, moderate resistance goes to gentamicin (40.68 %), and a low resistance to nitrofurantoin . similarly, *Klebsiella spp.* and *Proteus spp.* were vulnerable respectively to nitrofurantoin & gentamycin only but all other antimicrobial agents belong to low frequency of susceptibility (< 50 %). *Enterococcus spp* shows minimum resistance to nitrofurantoin, but moderately high resistance against penicillin, ciprofloxacin, norfloxacin & gentamycin. *Pseudomonas spp.* was vulnerable to amikacin, ciprofloxacin & norfloxacin only and showed 63% -83% resistance to all other first line drug. *Acinetobacter anitratus* was 80 % sensitive to amikacin & 20% to nitrofurantoin but showed > 95% resistance to all remaining antimicrobials. In female UTI was found stronger than in male [11]. This result has been co -relative regarding the study from Nepal, India and other countries [11,12]. Urinary tract infection is rapidly rising as a significant community acquired and nosocomial bacterial infection. Moreover, antimicrobial resistance to various classes of antibiotics to uropathogens shows a major health problem in different parts of the. world [13]. Female patients are quite active in term of urine culture than the male patients. But, Significant microbial growth was higher in case of female. The reason behind it is the cause of Urethral opening, short urethra and complicated physiology especially during pregnancy [14]. In between the age group from 20–40 are sexually active which is the most important risk factor in young women. Certain types of contraceptives can also invite the risk of UTIs [14, 15]. All ESBL

positive *E. coli* strains were resistant to cefotaxime, ceftazidime and ceftriaxone. This result is common with the study done by Islam et al. [14,16]. All *E. coli* isolates were resistant to cefotaxime and ceftriaxone in similar study by Chander and Shrestha et al [17].

### **Conclusion**

We found the high degree of multidrug resistance among bacterial uropathogens. Specially, the amount of resistance to amoxicillin, co-trimoxazole, flouroquinolones and third-generation cephalosporins were higher and these antibiotics ought to be ignored. Existing uropathogens highlights the highest rate of vulnerability to nitrofurantoin, amikacin and gentamicin which provide much better antibiotic coverage and can be adapted for practical treatment of urinary tract infections.

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