*Sunsari Technical College Journal, Volume 1, Issue 1, October 2012*
*ISSN 2091-2102*

*Original Article*

# ANTIMICROBIAL RESISTANCE PATTERN OF ESCHERICHIA COLI ISOLATED FROM URINE SAMPLES IN PATIENTS VISITING TERTIARY HEALTH CARE CENTRE IN EASTERN NEPAL

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## ABSTRACT

**Background:** Urinary tract infection (UTI) is one of the commonest bacterial infections caused by microbial invasion of tissue lining the urinary tract. *Escherichia coli* is the primary etiologic agent among both outpatients and inpatients. Antimicrobial resistance is an evolving and growing problem in UTI. Of more concern is increasing incidence of infections caused by strains of *E. coli* that are resistant to commonly used antimicrobial agents. **Objective:** The study was conducted with the objective to monitor the prevalence of E. coli and to assess the trend of drug resistance in patients visiting Nobel Medical College, Biratnagar, Nepal. **Materials and Methods:** Specimens were collected aseptically and cultured in bacteriological culture media and identified by using standard microbiological techniques as described in Bergey's Manual of systemic bacteriology. Antimicrobial susceptibility test was performed by modified Kirby-Bauer disk diffusion method. **Results:** Of 508 non-repeated urine samples the total growth was 18.30% (n=93). *E. coli* accounted for 86.02% (n=80). Highest samples were belonged to the age group 21-40 years and least samples belonged to the age group >61.Of total antibiotics used, amikacin showed the highest susceptibility of 96.25% followed by nitrofurantoin, gentamicin, cefotaxime and ceftazidime with 92.5%, 77.5%, 72.5% and 71.25% respectively among the isolates. Least susceptibility was shown by nalidixic acid with 22.5%. About half of the isolates were susceptible to norfloxacin and ciprofloxacin while no significant differences were seen among susceptibility to different antibiotics and age groups.Total multiple drug resistance (MDR) was 38.75% (n=31) with highest isolates belonged to the age group 21-40 years and least belonged to the age group <20 years. However, no significant difference was seen between MDR and age groups. The MDR was higher in male, 59.1% (13/22) than in female, 31.03% (18/58) which was statistically significant (p=0.021) showing about three times more MDR in male than in female. **Conclusion:** This study found more than one third of the urinary *E. coli* isolates were MDR. Higher resistance was seen to fluroquinolones and about one third to third generation cephalosporins. This necessitates a reevaluation of first and second line therapies for the treatment of UTI and regular monitoring of the usage of antimicrobials is done so as to optimize the empirical therapy.

**Key Words:** UTI, *E. coli*, Antimicrobial resistance, MDR

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## INTRODUCTION

Urinary tract infection (UTI) is one of the commonest domiciliary and nosocomial bacterial infections caused by microbial invasion of tissue lining the urinary tract. It refers to the presence of significant bacteriurea and pyurea in the mid-stream sample of urine.[1,2] The pathogens causing UTIs are almost always predictable, with E. coli being the primary etiologic agent among both outpatients and inpatients.[3,4,5] E. coli causes approximately 85 percent of cases of urethrocystitis, about 80 percent of cases of chronic bacterial prostatitis, and up to 90 percent of cases of acute pyelonephritis.[6] Antimicrobial resistance is an evolving and growing problem in UTI. Of more concern is increasing incidence of infections caused by strains of E. coli that are resistant to commonly used antimicrobial agents especially to trimethoprim-sulphamethoxazole (TMP/SMX) and beta-lactam antibiotics.[7] Therefore it is necessary for continuous surveillance of antimicrobial resistance among these organisms. The purpose of this study was to monitor the prevalence of E. coli and to assess the level of drug resistance in patients visiting Nobel Medical College, Biratnagar, Nepal.

## MATERIALS AND METHODOLOGY

The present study was conducted in patients visiting Nobel Medical College Teaching Hospital (NMCTH) Microbiology laboratory, from January to March 2011. This study included patients of age 33.56±20.454 years including male female patients 122 and 386, respectively.

A total of 508 subjects were enrolled, and routine culture and antibiotic susceptibility testing were performed using standard protocols. The identification of various gram negative isolates were done by using standard microbiological techniques, which comprises of studying the colonial morphology, staining reactions and various biochemical properties.[8] Isolated colonies from the pure culture were identified by performing the standard biochemical tests including catalase, oxidase, indole, methyl red, voges-prokauer, citrate, Triple sugar iron (TSI), urease and motility. Susceptibility tests of the different clinical isolates towards various antibiotics amikacin, gentamicin, norfloxacin, nitrofurantoin, nalidixic acid, ciprofloxacin, cefotaxime and ceftazidime (Hi Media, India) were performed by modified Kirby-Bauer disk diffusion method.[9]

Data was entered in MS Excel 2007, descriptive and inferential statistics were applied using SPSS. Chi-square test was used to determine significant association of dependant variables at 5% level of significance.

## RESULTS

A total of 508 non-repeated urine samples were collected, of which 18.30% (n=93) showed the significant bacteriurea. Of total isolates, E. coli accounted for 86.02% (n=80). Highest samples were belonged to the age group 21-40 years i.e. 54.33% (n=276) with growth of 13.04% (n=36) and least samples belonged to the age group >61 i.e. 8.07% (n=41) with growth of 29.26% (n=12).

Of total antimicrobials used, amikacin showed the highest susceptibility of 96.25% followed by nitrofurantoin, gentamicin, cefotaxime and ceftazidime with 92.5%, 77.5%, 72.5% and 71.25% respectively. Least susceptibility was shown to nalidixic acid with 22.5% while about half of the isolates were susceptible to norfloxacin and ciprofloxacin.

On gender wise distribution of antibiotic susceptibility pattern, highest susceptibility was seen for Amikacin with 90.90% and 98.30% in male and female which was statistically insignificant (p=0.182). Norfloxacin (p=0.001), nitrofurantion (p=0.005), nalidixic acid (p=0.014) and ciprofloxacin (p=0.02) were significantly different among male and female with higher susceptibility seen among female than male.

**Table 1: Gender wise distribution of antibiotic sensitivity pattern**

| Antibiotics | Male | | Female | | Total | | P value |
|---|---|---|---|---|---|---|---|
| | Resistant (%) | Sensitive (%) | Resistant (%) | Sensitive (%) | Resistant (%) | Sensitive (%) | |
| Amikacin | 2(9.10) | 20(90.90) | 1(1.70) | 57(98.30) | 3(3.9) | 77(96.10) | 0.182 |
| Gentamicin | 9(40.90) | 13(59.10) | 9(15.50) | 49(84.50) | 18(22.50) | 62(77.50) | 0.19 |
| Norfloxacin | 18(81.80) | 4(18.20) | 24(41.40) | 34(58.60) | 42(52.50) | 38(47.50) | 0.001 |
| Nitrofurantoin | 5(22.70) | 17(77.30) | 1(1.70) | 57(98.30) | 6(7.50) | 74(92.50) | 0.005 |
| Nalidixic acid | 21(95.50) | 1(4.50) | 41(70.70) | 17(29.30) | 62(77.50) | 18(22.50) | 0.014 |
| Ciprofloxacin | 16(72.70) | 6(27.30) | 20(34.50) | 38(65.50) | 36(45.00) | 44(55.00) | 0.02 |
| Cefotaxime | 8(36.40) | 14(63.60) | 14(24.10) | 44(75.90) | 22(27.50) | 58(72.50) | 0.206 |
| Ceftazidime | 8(36.40) | 14(63.60) | 15(25.90) | 43(74.10) | 23(28.75) | 57(71.25) | 0.255 |

Of total isolates, 38.75% (n=31) were multiple drug resistant (MDR) with highest isolates belonged to the age group 21-40 years i.e. 51.61% (n=16) and least belonged to the age group <20 years, 16.13% (n=5). Among isolates obtained from different age groups, highest MDR belonged to the age group > 60 years, 50% (6/12) followed by age group 21-40 years, 44.44% (16/36). However, no significant difference was seen between MDR and age groups (p=0.264).

**Table 2: Multiple drug resistance among different age groups**

| | MDR | Non-MDR | Total | p-value |
|---|---|---|---|---|
| **Age_group** < 20 | 5 | 15 | 20 | |
| 21-40 | 16 | 20 | 36 | |
| 41-60 | 4 | 8 | 12 | 0.264 |
| >61 | 6 | 6 | 12 | |
| **Total** | 31 | 49 | 80 | |

Of total MDR, female and male accounted for 58.06% and 41.97% respectively. Among total isolates, the MDR was higher in male, 59.1% (13/22) than in female, 31.03% (18/58) which was statistically significant (p=0.021) showing about three times more MDR in male than in female.

**Table 3: Genderwise distribution of Multiple drug resistance**

| | | MDR | Non-MDR | Total | p-value |
|---|---|---|---|---|---|
| **Sex** | **Male** | 13 | 9 | 22 | |
| | **Female** | 18 | 40 | 58 | 0.021 |
| **Total** | | 31 | 49 | 80 | |

## DISCUSSION

This study was conducted with the aim to determine the status of multiple drug resistance E. coli isolated from urine samples. Altogether 508 samples were collected of which the overall growth was 18.3%. The low growth might be due to inclusion of every patients requesting for culture regardless of their symptoms and illness or prior use of antibiotics or might be due to presence of fastidious organisms that we are not be able to grow on routine culture media.[10] *E. coli* accounted for more than four fifth of the total isolates. Such higher rates might be due to endogenous source of infection as they are the most frequent isolates at hospital acquired and community acquired infections.[2,7,10,11]

More than half of the samples belonged to the age group 21-40 years with growth of 13.04% with higher in females than in males. The females of this age group are sexually active and are of child bearing age. A number of studies suggested that sexual activity is one of the important influential factors for UTI in women.[2,8,12]

The growth was higher in the age group >61 years and accounted for about one third of total samples processed. This finding suggests that higher chance of acquiring infections with increasing age that might be due to deterioration of immunity with age.[2]

Amikacin (96.25%) followed by nitrofurantoin, gentamicin, cefotaxime and ceftazidime with 92.5%, 77.5%, 72.5% and 71.25% respectively showed susceptibility among the isolates. Such higher susceptibility were seen in different works.[1,9,12,13,15] Nearly half of the isolates were resistant to norfloxacin and ciprofloxacin. This might be due to unregulated use of these antibiotics at community level.[5,7,13,14] However, no significant associations were seen with susceptibility to antibiotics with age groups and gender.

More than one third of the isolates were multiple drug resistant (MDR) with highest isolates belonged to the age group 21-40 years and least belonged to the age group <20 years. About half MDR was observed from age group >60 years followed by age group 21-40 years. This might suggests that increase in drug resistance might be due to more exposure to antibiotics with increasing age which results in high MDR positivity.[17,18,19,20] However, no significant difference were seen between MDR and age groups.

Among total isolates, the MDR was higher in male of more than half than in female of about one third which was statistically significant showing about three times more MDR in male than in female. This might suggests that in female the infection is more commonly endogenous that might be a normal flora but in case of male there is more chance of exogenous infections and are most commonly a pathogens rather than normal flora that are more resistant to the drugs.[2]

## CONCLUSION

In conclusion, this study revealed the magnitude of the problems of multiple drug resistance among E. coli isolated from urine samples. More than one third of the isolates were MDR. Higher resistance was seen to fluroquinolones and about one third to third generation cephalosporins. This necessitates a reevaluation of first and second line therapies for the treatment of urinary tract infections due to the organisms and regular monitoring of the usage of antimicrobials is done so as to minimize the complications in the case treatment.

## ACKNOWLEDGEMENTS

We wish to express our sincere gratitude to all the staff of Department of Microbiology, Nobel Medical College for their kind cooperation. We are also grateful to the Central Department of Microbiology, Tribhuvan University for all the help provided.

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