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*Full Length Research Paper*

# Urinary tract infections in Kidney transplant patients of Kathmandu Valley

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**A laboratory based study was carried out with the objective to isolate the bacteria causing UTI in kidney transplanted and other UTI suspected patients and find out their antibiotic susceptibility pattern. A total of 1233 urine samples (439 urine samples from kidney transplanted patients) were collected and subjected to laboratory analysis and culture. The prevalence of uropathogens was found to be 14.19% (175/1233). Out of total 175 isolates of uropathogens, 16 different bacterial species were identified, of which 94.29% (165/175) gram negative bacteria were belonging to 12 different species. In gram negative the most predominant was *Escherichia coli* (64.14%), followed by *Klebsiella pneumoniae* (12.57%), *Klebsiella oxytoca* (5.14%), *Acinetobacter spp.* and *Pseudomonas aeruginosa* (3.43%). Out of total, 439 urine samples from Kidney transplanted patients, only 22 samples (5.01%) had showed significant growth. The most efficient first line antibiotics for isolates was found to be Ceftriaxone 68.57%, followed by Nitrofurantoin 60% and in second line antibiotics Ceftazidime –clavunic acid and Amikacin showed susceptibility of 89.55%. Out of 175 uropathogens, 48% (84/175) isolates were found to be MDR positive. In gram negative bacteria, *E. coli* showed highest percentage of MDR that is 53.27%. Association of significant bacteriuria and gender of patients was found to be statistically significant ($p<0.05$). Transplantation status and infection status were found to have strong association ($p<0.001$).**

**Key words:** Uropathogens, Kidney transplanted patients, MDR, bacteriuria, antibiotics.

## INTRODUCTION

The term Urinary tract infection (UTI) refers to the invasion of the urinary tract by a non resident infectious organisms. Kass (1956), gave a criterion of active bacterial infection of urinary tract according to which, a count exceeding $10^5$ organisms per ml denotes significant bacteriuria and indicates active UTI. Contamination accounts for less than $10^4$ organisms per ml and usually less than $10^3$ per ml (Arora, 2004). UTI is one of the most important causes of morbidity in the general population and it is the second most common cause of hospital visits. Recurrent infections are common and can lead to irreversible damage of kidneys, resulting in renal hypertension and renal failure in severe cases. In the community, women are more prone to develop UTI. About 20% of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per year. Pregnancy also makes them more susceptible to infections (Das et al., 2006). UTI are important complications of diabetes and renal diseases, renal transplantation and structural and neurological abnormalities that interfere with urine flow. In 40% to 60% of renal transplant recipient, the urinary tract is the source of bacteria and in these patients recurrence is about 40% (Forbes et al., 2002). Kidney transplantation originated in the United States in 1954. In developed countries, approximately 75% of the transplants performed use organs from cadaveric donors while the developing countries transplant about 85-100% of the kidneys from living donors (Enns and Aryal, 2011). UTI is the most common post transplantation infection. Nearly, 80% of renal transplant recipient suffer at least one episode of infection during the first year after transplantation and infection remains the leading cause of morbidity and mortality throughout the post transplant course (Charfeddine et al., 2002). It is estimated that about 2.7 million people are suffering from kidney disease in Nepal and about two thousands add up to this

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number yearly. It is further estimated that nearly 750 kidney transplantation are needed in a year to meet the national needs, which accounts to about two transplantations a day. But due to various constraints prevalent, less than ten transplantations altogether in the two institutions, are being performed in a month. Nepali law only permits the transplantation among close relatives and potential kidney donor's can be the father, mother, sister, brother, husband, wife, son, daughter, uncle, aunt, mother-in-law, father in-law, step father/mother or adopted children. This strict legislation is to prevent the possible organ trade and foul play in procuring the organ. (Chalise et al., 2010). This study aims to investigate the bacterial pathogens responsible for UTI in kidney transplanted patients and compare with UTI in other patients (other than kidney transplanted patients) and find out their antibiotic susceptibility pattern in patients visiting tertiary level reference laboratory, National Public Health laboratory (NPHL), Teku Kathmandu.

## MATERIALS AND METHODS

The study was carried out from April 2010 to January 2011. During this period, a total of 1233 urine samples from patients suspected of UTI were collected and processed according to the standard laboratory methods in National Public Health Laboratory Teku (Vandepitte et al., 2003).

### Specimen Collections

Each patient was instructed for proper collection of sample. The patients were given a clean, dry and sterile leak proof container and requested for 5-10 ml mid-stream urine sample.

### Specimen Evaluation

Before proceeding, the urine specimens were evaluated in terms of their acceptability. Considerations included proper labeling, visible signs of contamination and any transportation delays in getting the specimen to the laboratory. A properly labeled specimen contained patient's full name, date and time of collection. Single urine specimen was collected from each patient so bacteriological culture was performed first followed by the routine microscopic observation.

### Sample Processing

**Routine Macroscopic Examination:** Macroscopic examination of the urine sample collected was conducted by observing its color and appearance and reported accordingly (Vandepitte et al., 2003; Collee et al*.,* 2001).

**Routine Microscopic Examination:** About 5 ml (about half) of urine sample was taken in a clean sterile centrifuge tube and centrifuged at 3000 rpm for 10 min. The supernatant was discarded. The sediment was then examined by wet mount preparation.

**Wet Mount Preparation:** Microscopic examination of urinary sediments by wet mount includes the detection of White Blood Cells (pus cells) and Red Blood Cells. Number of WBC and RBC were estimated as number per High Power Field that is, number of objects seen in 40X objective of microscope.

### Culture of Specimen

Semi-quantitative culture technique was used to culture urine specimens and to detect the presence of significant bacteriuria by standard methods (Vandepitte et al., 2003; Collee et al., 2001).

An inoculating loop of standard dimension was used to take up approximately fixed (±10% error was accepted) and known volume (0.001ml) of mixed uncentrifuged urine was inoculated on the surface of 5% Blood Agar (BA) and MacConkey Agar (MA). Urine specimen was thoroughly mixed to ensure uniform suspension of bacteria before inoculating the agar plates. The inoculated MA and BA plates were aerobically incubated overnight at 37ºC.

The bacterial count was reported as: Non Significant; Less than $10^4$ organisms/ml, Significant Bacteriuria; More than $10^5$ organisms/ml, Repeat specimen; $10^4$-$10^5$ organisms/ml.

### Identification of Isolates

The isolated colony from plates showing significant growth was further preceded for identification. Plate showing no growth, mixed growth and bacterial growth of insignificant number was excluded from the study. Identification was conducted according to the protocol (Cheesbrough, 1984). The single distinct colony was gram stained. A single distinct colony from Mac Conkey Agar for both the gram negative and gram positive bacteria was picked by using sterile straight wire loop and inoculated on Nutrient Agar and incubated at 37ºC for 24 hours. After the overnight incubation, the culture was used to perform biochemical test and antibiotic susceptibility test.

### Antibiotic Susceptibility Testing

The antimicrobial susceptibility testing of the isolates were done by modified Kirby-Bauer disc diffusion method

**Table 1.** Transplantation status of patients and their culture positivity.

| Age group | KidneyTransplanted | | Non kidney transplanted | | Total | % |
|---|---|---|---|---|---|---|
| | Positive | % | Positive | % | | |
| 0-10 | 0 | 0 | 2 | 1.31 | 2 | 1.31 |
| 10-20 | 1 | 4.56 | 7 | 4.58 | 8 | 9.14 |
| 20-30 | 4 | 18.18 | 53 | 34.64 | 57 | 52.82 |
| 30-40 | 5 | 22.73 | 43 | 28.10 | 48 | 50.83 |
| 40-50 | 5 | 22.73 | 18 | 11.76 | 23 | 34.49 |
| 50-60 | 5 | 22.73 | 19 | 12.42 | 24 | 35.15 |
| 60-70 | 2 | 9.09 | 7 | 4.58 | 9 | 13.67 |
| >70 | 0 | 0 | 4 | 2.61 | 4 | 2.61 |
| Total | 22 | 100 | 153 | 100 | 175 | |

**Table 2.** Distribution of isolated organisms in urine culture.

| S.N | Organisms isolated | Non-Kidney transplanted | % | Kidney Transplanted | % | Total | % |
|---|---|---|---|---|---|---|---|
| 1. | *Enterobacter spp* | 3 | 1.96 | 0 | - | 3 | 1.71 |
| 2. | *Acinetobacter spp* | 5 | 3.27 | 1 | 4.55 | 6 | 3.43 |
| 3. | *Alkaligenes spp* | 1 | 0.65 | 0 | - | 1 | 0.57 |
| 4. | *Citrobacter freundii* | 3 | 1.96 | 0 | - | 3 | 1.71 |
| 5. | *Escherichia coli* | 98 | 64.05 | 9 | 40.91 | 107 | 61.14 |
| 6. | *Edwardsiella spp* | 1 | 0.65 | 0 | - | 1 | 0.57 |
| 7. | *Enterococci spp* | 3 | 1.96 | 1 | 4.55 | 4 | 2.29 |
| 8. | *Klebsiella oxytoca* | 7 | 4.58 | 2 | 9.09 | 9 | 5.14 |
| 9. | *Klebsiella pneumoniae* | 17 | 11.11 | 5 | 22.73 | 22 | 12.57 |
| 10. | *Proteus mirabilis* | 3 | 1.96 | 1 | 4.55 | 4 | 2.29 |
| 11. | *Proteus vulgaris* | 1 | 0.65 | 1 | 4.55 | 2 | 1.14 |
| 12. | *Pseudomonas aeruginosa* | 4 | 2.61 | 2 | 9.09 | 6 | 3.43 |
| 13. | *Staphylococcus aureus* | 2 | 1.31 | 0 | - | 2 | 1.14 |
| 14. | *Staphylococcus saprophyticus* | 2 | 1.31 | 0 | - | 2 | 1.14 |
| 15. | *Streptococcus spp* | 2 | 1.31 | 0 | - | 2 | 1.14 |
| 16. | *Providencia spp* | 1 | 0.65 | 0 | - | 1 | 0.57 |
| | Total | 153 | | 22 | | 175 | 100 |

as recommended by CLSI (Clinical and Laboratory Standards Institute) using Mueller Hinton agar (MHA).

## Quality Control

Strict quality control was maintained to obtain reliable microbiological results. The quality of each agar plate prepared was maintained by incubating one plate of each batch in the incubator. Control strains of ATCC were used for the identification test and for the standardization of Kirby- Bauer test and also for correct interpretation of inhibition zones of diameter. Quality of sensitivity test was maintained by maintaining the thickness of MHA at 4mm and the pH 7.2-7.4. Similarly antibiotics disks having

**Table 3.** Antibiotic susceptibility pattern of isolated organisms.

| Antibiotics | Gram negative | | | Gram positive | | | Total | % |
|---|---|---|---|---|---|---|---|---|
| | Susceptible | % | Total | Susceptible | % | Total | Susceptible | Susceptible |
| Amoxicillin | 43 | 26.06 | | 6 | 60 | | 49 | 28 |
| Ceftriaxone | 112 | 67.88 | | 8 | 80 | | 120 | 68.57 |
| Ofloxacin | 91 | 55.15 | 165 | 2 | 20 | 10 | 93 | 53.14 |
| Ciprofloxacin | 86 | 52.12 | | 4 | 40 | | 90 | 51.43 |
| Norfloxacin | 85 | 51.52 | | 4 | 40 | | 89 | 50.86 |
| Cotrimoxazole | 67 | 40.61 | | 8 | 80 | | 75 | 42.86 |
| Nitrofurantoin | 101 | 61.21 | | 4 | 40 | | 105 | 60 |
| **Second generation antibiotics** | | | | | | | | |
| Antibiotics | Susceptible | % | Total | Susceptible | % | Total | Susceptible | Susceptible |
| Ceftazidime | 29 | 43.94 | | 0 | - | | 29 | 43.94 |
| Ceftazidime clavunic acid | 60 | 90.91 | 66 | 0 | - | - | 60 | 90.91 |
| Amikacin | 60 | 90.91 | | 0 | - | | 60 | 90.91 |
| Gentamycin | 31 | 46.97 | | 0 | - | | 31 | 46.97 |
| Cephipime | 22 | 33.33 | | 0 | - | | 22 | 33.33 |
| Penicillin | 0 | | - | 3 | 50 | 6 | 3 | 50 |
| Oxacillin | 0 | - | - | 5 | 83.33 | | - | 83.33 |
| Total | 675 | | | 39 | | | 844 | |

correct amount as indicated was used. Strict aseptic condition was maintained while carrying out all the procedures.

## RESULTS

Among 1233 urine sample cultured, a total of 175 uropathogens belonging to 16 different species were isolated (Table 2). Out of 439 urine samples of kidney transplanted patients, 22 samples had showed growth of bacteria (Table 1). Altogether, 14.19% urine samples had showed growth of bacteria, of which 94.29% (165/175) were gram negative uropathogens. Among gram negative, *E. coli* was the major isolates. Ceftriaxone showed the susceptibility of 68.57% (120/175) among total isolates, followed by Nitrofurantoin 60% (105/175) in 1st generation antibiotics. In second generation antibiotics, Ceftazidime-clavunic acid and Amikacin showed the susceptibility of 90.91% (60/66) and Oxacillinc showed the susceptibility of 83.33% (5/6) among gram positive isolates (Table 3). Out of 175 positive cases, 48% isolates were multiple drug resistant (MDR) that is (84/175) and MDR in *E. coli* were found to be 53.27% (57/107). Among 107 *E. coli* isolates 57 (53.27%) were MDR (Table 4). This study showed the significant association between infection among Kidney transplanted and Other UTI suspected patients ($p<0.05$). But there was no any significant association between MDR status between Kidney- transplanted and other UTI suspected patients.

## DISCUSSION

Out of total 1233 urine samples, 14.19% (n=175) samples showed significant growth. A similar study carried out by Chhetri et al. (2001) showed growth positi-

**Table 4.** Distribution of MDR isolates.

| S.N | Organisms isolated | Total organisms isolated | Multidrug resistant | % |
|---|---|---|---|---|
| 1. | *Enterobacter spp* | 3 | 1 | 33.33 |
| 2. | *Acinetobacter spp* | 5 | 1 | 0.2 |
| 3. | *Alkaligenes spp* | 1 | - | - |
| 4. | *Citrobacter freundii* | 3 | 1 | 33.33 |
| 5. | *E.coli* | 107 | 57 | 53.27 |
| 6. | *Edwardsiella spp* | 1 | - | - |
| 7. | *Enterococcus spp* | 4 | 3 | 75 |
| 8. | *Klebsiella oxytoca* | 8 | 2 | 25 |
| 9. | *Klebsiella pneumonia* | 18 | 8 | 44.44 |
| 10. | *Proteus mirabilis* | 6 | 4 | 66.67 |
| 11. | *Proteus vulgaris* | 2 | 2 | 100 |
| 12. | *Pseudomonas aeruginosa* | 6 | 5 | 83.33 |
| 13. | *Staphylococcus aureus* | 2 | - | - |
| 14. | *Staphylococcus saprophyticus* | 2 | 1 | 50 |
| 15. | *Streptococcus spp* | 2 | - | - |
| 16. | *Providencia spp* | 1 | - | - |
| | *Total* | 175 | 84 | 48 |

vity of 21.8% (Chhetri, et al., 2001). The low growth positive rate observed in this study might be due to inclusion of kidney transplant patients and others for routine check up only. This might also be due to inclusion of samples from patients under treatment. Among 1233 total sample, 64.40% (794/1233) were from non kidney transplanted, whereas 35.60% (439/1233) were kidney transplanted patients. Only 12.57% (n=22) kidney transplanted patients had significant bacteriuria. Among total kidney transplanted patients, 75.85% (333/439) were male patients. Among male patients 63.64% (14/22) were infected, whereas 36.36% (8/22) was female. Similar study done by Ghimire et al. (2004), 73.0% males and 27.0% females had significant bacteriuria (Ghimire and Sharma, 1995). Altogether 16 different bacterial isolates were found in this study. Among the isolates, *E. coli* (61.14%) was found to be the most predominant organism followed by *Klebsiella pneumoniae* (12.57%), *Klebsiella oxytoca* (5.14%). Higher prevalence of *E. coli* was found in this study also resembled the study done by various other workers viz: (Chhetri et al., 2001); Sharma *et al*., 1983); (Jha and Yadav, 1992). The result is also in harmony with the study done at international context: (Kahlmeter, 2000); (Farrell et al., 2003). In this study, in case of first generation antibiotics Ceftriaxone (68.57%) was effective against isolated organisms followed by Nitrofurantoin (60%) of susceptibility. In second generation antibiotics Ceftazidime-clavunic acid amd Amikacin (91.91%) showed similar susceptibility towards gram negative isolates which were resistant to first generation antibiotics. Similar study performed by Jha and Bapat (2005) at Sukraraj Tropical Hospital, 92.5% of urinary isolates were susceptible to Aminoglycosides groups of antibiotics. About 72% isolated organisms were resistant to Ampicillin, the least effective drug against gram negative bacteria, followed by Cotrimoxazole (42.56%). Quinolone/Fluroquinolones groups of antibiotics were showed susceptibility in similar manner, Ofloxacin, Ciprofloxacin and Norfloxacin was showed susceptibility of 53.14%, 51.43% and 50.86% respectively. In the urine isolates, Amoxicillin (28.04%) was found the least susceptible towards *E. coli* followed by Cotrimoxazole (36.36%). Nitrofurantoin (68.22%) was found to be most efficient antibiotics followed by Ceftriaxone (66.36%). Ofloxacin (49.53%), Ciprofloxacin (46.73%) and Norfloxacin (43.92%) showed susceptibility in Fluroquinolones group of antibiotics. The study conducted by Karki et al. (2004) among outpatient and inpatient of Kathmandu Medical College Teaching Hospital, the *E. coli* isolates was most susceptible to Nitrofurantoin. The similar study conducted by (Arosio et al., 1978) and (Obi et al., 1996) resistant to Amoxicillin was observed. In second generation antibiotics *E. coli* were most susceptible to Ceftazidime -clavunic acid and Amikacin (91.67%). In this study, total multiple drug resistant (MDR) cases were 48% (84/175). Among total cases, MDR in *E. coli* were found to be 53.27% (57/107). Higher rate of MDR was found in Kidney transplanted

patients 59.09% (13//22) than in other patients 46.41% (71/153).

Among 1233 urine sample cultured, a total of 175 uropathogens belonging to 16 different species were isolated. Gram negative uropathogens 94.29% (165/175) were found predominant. Among Gram negative, *E. coli* was the major isolates. This study showed the significant association between infection among Kidney transplanted and Non transplanted patients ($p<0.05$). But there was no significant association between MDR status between Kidney-transplanted and Other UTI suspected patients.

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