Comparative evaluation of urine isolates among kidney transplanted and other UTI suspected patients visiting National Public Health Laboratory, (NPHL) Teku, Nepal

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Abstract
Objective: This study compare the prevalence of urinary tract infection among kidney transplanted and other UTI suspected patients visiting National Public Health Laboratory, Teku, Nepal.

Materials and Methods: The study was carried out from January 2011 to October 2011. During this period, a total of 1233 urine samples from patients suspected of UTI were collected and processed. The antimicrobial susceptibility was tested by the modified Kirby-Bauer’s disc diffusion method as per the CLSI guidelines. All the data obtained was statistically analyzed by using Statistical Package for Social Sciences (SPSS) version 16.

Results: Prevalence of uropathogens was found to be 14.19% (175/1233). 16 species of different bacterial isolates were isolated, identified and among them, 94.29% (165/175) were of Gram negative and 5.71% (10/175) of Gram positive organisms. Out of total 439 Kidney transplanted patients, only 5.01% (22) of urine sample showed significant growth. The most efficient first line antibiotic for isolates was found to be Ceftriaxone 68.57%, and in second line antibiotics Ceftazidime-clavunic acid and Amikacin showed better efficacy. In the total 175 uropathogens, 48% (84/175) isolates were found to be MDR isolates.

Conclusion: A 14.19% prevalence of UTIs was found. 94.29% (165/175) of Gram negative uropathogens were found to be predominant. This study showed the significant association between infection among Kidney transplanted and Non-transplanted patients (p<0.05).

Keywords: Urinary Tract Infection, Kidney transplant, MDR

1. Introduction

Urinary tract infection (UTI) is one of the commonest domiciliary and nosocomial bacterial infections comprising of a variety of clinical conditions caused by microbial invasion of tissue lining of the urinary tract, which extends from renal cortex to the urethral meatus.19. Urinary tract infection (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 infections6.
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%.

2. Materials and Methods

The study was carried out from January 2011 to October 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 NPHL, Teku, Kathmandu.

2.1 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.

2.2 Specimen evaluation

Before proceeding, the urine specimens were evaluated in terms of their acceptability. Considerations included proper labelling, visible signs of contamination and any transportation delays in getting the specimen to the laboratory. A properly labelled 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.

2.3 Sample processing

2.3.1 Routine macroscopic examination

Macroscopic examination of the urine sample collected was conducted by observing its color and appearance and reported accordingly.

2.3.2 Routine microscopic examination

About 5 ml (about half) of urine sample was taken in a clean sterile centrifuge tube and centrifuged 1t 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 WBC (pus cells) and RBC. Number of WBC and RBC were estimated as number per HPF i.e., number of objects seen in 40X objective of microscope.

2.4 Culture of specimen

Semi-quantitative culture technique was used to culture urine specimens and to detect the presence of significant bacteriuria by standard methods. 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 Mac Conkey 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:

| Count Description | Organisms/ml |
|-------------------|--------------|
| Insignificant     | Less than 10⁴ |
| Significant       | More than 10⁵ |
| Repeat            | 10⁴-10⁵      |

2.5 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 provided by Collee. The single distinct colony was gram stained. A single distinct colony from MA for both the gram negative and gram positive bacteria was picked by using sterile straight wire loop and inoculated on NA. It was incubated at 37ºC for 24 hours. After the overnight incubation, the culture was used to perform biochemical test and antibiotic susceptibility test.

2.5.1 Antibiotic susceptibility testing

The antimicrobial susceptibility testing of the isolates were done by modified Kirby-Bauer disk diffusion method as recommended by CLSI (Clinical and Laboratory Standards Institute) using Mueller Hinton agar (MHA).

MDR isolates were defined as those isolates resistant to three or more group of antimicrobial agents. Among the 175 isolates that were evaluated against 14 antimicrobials, 7 were first line antibiotics whereas remaining were second line antibiotics according to laboratory policy.
3. Results

Out of total 1233 sample collected only 14.19% of the cases showed growth of bacteria. 175 uropathogens belonging to 16 different species were isolated. Gram negative uropathogens constituted 94.29% (165/175). Among Gram negative, \textit{E.coli} was the major isolates. Gram positive uropathogens were found in 5.71% (10/175) of cases among them \textit{Enterococcus} spp were predominant. There is significant difference of positive growth between male and female patients (\(P<0.05\)) and this study shows the significant association between infection among Kidney transplanted and Non-transplanted patients (\(P<0.05\)). But there was no any significant association between MDR status between Kidney-transplanted and Non-transplanted patients. Ceftriaxone was found to be most effective antibiotic, followed by Nitrofurantoin in 1\textsuperscript{st} line antibiotics. In second line antibiotics, Ceftazidime-clavunic acid and Amikacin showed efficacy against 90.91% (60/66) of isolates, and Oxacillin showed the better efficacy against gram positive isolates. Multiple drug resistant (MDR) cases were 48 % (84/175), and MDR in \textit{E. coli} were found to be 53.27% (57/107). Similarly \textit{Proteus vulgaris} were 100% MDR.

| Table 1. Age and gender wise distribution of culture positive results |
|-----------------|-------|-------|-------|
| Age group       | Male  | Female | Total |
|                 | Number | %    | Number | %    | Number | %    |
| 0-10            | 1      | 0.57 | 1      | 0.57 | 2      | 1.14 |
| 10-20           | 4      | 2.29 | 4      | 2.29 | 8      | 4.58 |
| 20-30           | 17     | 9.71 | 40     | 22.86| 57     | 32.57|
| 30-40           | 24     | 13.71| 24     | 13.71| 48     | 27.43|
| 40-50           | 12     | 6.86 | 11     | 6.29 | 23     | 13.15|
| 50-60           | 10     | 5.71 | 14     | 8    | 24     | 13.71|
| 60-70           | 5      | 2.86 | 4      | 2.29 | 9      | 5.15 |
| >70             | 1      | 0.57 | 3      | 1.71 | 4      | 2.28 |
| Total           | 74     | 42.29| 101    | 57.71| 175    | 100  |

| Table 2: Distribution of urine isolates: |
|----------------------------------------|
| S.N | Organisms                  | Number of Isolates |
|-----|----------------------------|--------------------|
| 1.  | \textit{Enterobacter} spp | 3                  |
| 2.  | \textit{Acinetobacter} spp | 6                  |
| 3.  | \textit{Alkaligenes} spp   | 1                  |
| 4.  | \textit{Citrobacter freundii} | 3                  |
| 5.  | \textit{Escherichia coli}  | 107                |
| 6.  | \textit{Edwardsiella} spp  | 1                  |
| 7.  | \textit{Enterococci} spp   | 4                  |
| 8.  | \textit{Klebsiella oxytoca} | 9                  |
| 9.  | \textit{Klebsiella pneumoniae} | 22                |
| 10. | \textit{Proteus mirabilis}  | 4                  |
| 11. | \textit{Proteus vulgaris}   | 2                  |
| 12. | \textit{Pseudomonas aeruginosa} | 6                |
| 13. | \textit{Staphylococcus aureus} | 2                 |
| 14. | \textit{Staphylococcus saprophyticus} | 2       |
| 15. | \textit{Streptococcus} spp | 2                  |
| 16. | \textit{Providencia} spp   | 1                  |
|     | Total                      | 175                | 100   |
Table 3. Antibiotic susceptibility pattern of isolated organisms

| Antibiotics      | Gram negative |          | Gram positive |          | Total |          |
|------------------|---------------|----------|---------------|----------|-------|----------|
|                  | Susceptible % | Total    | Susceptible % | Total    | Susceptible | %       |
| Amoxicillin      | 26.06         | 43       | 60            | 6        | 49    | 28       |
| Ceftriaxone      | 67.88         | 112      | 80            | 8        | 120   | 68.57    |
| Ofloxacin        | 55.15         | 91       | 20            | 2        | 93    | 53.14    |
| Ciprofloxacine   | 52.12         | 86       | 40            | 4        | 90    | 51.43    |
| Norfloxacine     | 51.52         | 85       | 40            | 4        | 89    | 50.86    |
| Cotrimoxazole    | 40.61         | 67       | 80            | 8        | 75    | 42.86    |
| Nitrofurantoin   | 61.21         | 101      | 60            | 4        | 105   | 60       |

Second generation antibiotics

| Antibiotics          | Susceptible % | Total | Susceptible | Total | Susceptible | %       |
|----------------------|---------------|-------|-------------|-------|-------------|---------|
| Ceftazidime          | 43.94         | 29    | -           | -     | 29          | 43.94   |
| Ceftazidime clavunic acid | 90.91     | 60    | -           | -     | 60          | 90.91   |
| Amikacin             | 90.91         | 60    | -           | -     | 60          | 90.91   |
| Gentamycin           | 46.97         | 31    | -           | -     | 31          | 46.97   |
| Cephipime            | 33.33         | 22    | -           | -     | 22          | 33.33   |
| Penicillin           | -             | 0     | -           | 3     | 3           | 50      |
| Oxacillin            | -             | 0     | -           | 5     | 5           | 83.33   |
| Total                | 675           | 39    | 844         |       |             |         |

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                         | 0                   | 0    |
| 4   | Citrobacter freundii | 3                        | 1                   | 33.33|
| 5   | E.coli             | 107                       | 57                  | 53.27|
| 6   | Edwardsiella spp   | 1                         | 0                   | 0    |
| 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                        | 0                   | 0    |
| 14  | Staphylococcus saprophyticus | 2            | 1                   | 50   |
| 15  | Streptococcus spp  | 2                         | 0                   | 0    |
| 16  | Providencia spp    | 1                         | 0                   | 0    |
| Total|                   | 175                       | 84                  | 48   |
4. Discussion

Out of total 1233 urine samples, 175 (14.19%) samples showed significant growth. A similar study carried out by Chhetri28 showed growth positivity of 21.8%. 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.

In this study, age group (20-30) years had got high prevalence of UTI, a total of 32.57% (57/175) of patients were found to have significant bacteriuria. In this study female of childbearing age group (20-40) had 36.57% (64/175) growth positive cases. Previous study done by Shrestha26, Steenberg28, Rajbhandari and Shrestha25 also found the similar results. This result suggests that sexually active and women of childbearing age are more susceptible to UTI. Females are more susceptible to UTI than males. Also in the present study, this fact was supported where the rate of growth positivity was found to be 57.38 % (101/176) in females and 42.62% (75/176) in males. This higher growth positivity seen in females was found to be statistically significant (p<0.05) and is attributed to their anatomical structure (short urethra and proximity to anal orifice) leading to easy access for coliform bacilli. This result confirms and expands the previous findings17,14,4,13,25.

In this study, age group of 20-40 years showed the highest percentage of growth positivity. High-infected females also belonged to the same group. This finding correlates to the results of Steenberg28, Manandhar20, Rajbhandari and Shrestha25, Regmi26, Shrestha27 and Jha and Bapat13. The females of this age group are sexually active and are of childbearing age. A number of studies suggest that sexual activity is an important factor in the pathogenesis of UTI in women. Prevalence of UTI in nuns and unmarried women is considerably lower than in married women13. These studies also support the fact that the sexual activities predispose an increase in incidence of UTI in sexually active ages. Among males, highest growth positivity was found among age group of 30-40 years. The similar study done by Jha N and Bapat13 also showed that the highest percentage of infected male was found in age group 31-40. Among 1233 total patients 64.40% (794/1233) were non kidney transplanted patients, whereas 35.60% (439/1233) were kidney transplanted patients. Among total kidney transplanted patients, significant bacteriuria was seen among only 22 cases (12.57%). The low growth might be due to prior use of antibiotic prophylaxis and another reason for low positivity may be due to low sample size for kidney transplanted patients21. Among total kidney transplanted patients 75.85% (333/439) were male patients, whereas only 24.15% (106/439) were female patients. Among male patients 63.64% (14/22) were infected, whereas 36.36% (8/22) were female patients. The study done by Ghimire11 showed that, 73.0% males and 27.0% females were found to be infected among kidney transplanted patients, and significant bacteriuria was seen only in 30 cases (15.0%). 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%) Acinetobacter spp (3.43%) and Enterococci (2.9%). Higher prevalence of E. coli seen in this study also resembled the study done in Nepal by various other authors4,26,14,20,7. The result is also in harmony with the study done at international context27,15,8,19,10,18.

In this study, among first line antibiotics, Ceftriaxone (68.57%) was effective against isolated organisms followed by Nitrofurantoin (60%). In second line antibiotics Ceftriaxime-clavuninc acid and Amikacin (91.91%) showed similar susceptibility towards gram negative isolates which were resistant to first generation antibiotics. Similar study performed by Jha and Bapat13 at Sukraraj Tropical Hospital, 92.5% of urinary isolates were susceptible to Aminoglycosides groups of antibiotics.

On the other hand, (72%) organisms were resistant to Ampicillin. Which was found to be the least effective drug against Gram negative bacteria, followed by Cotrimoxazole (42.56%). Quinolone/Fluroquinolones groups of antibiotics showed susceptibility in similar manner, Ofloxacin, Ciprofloxacin and Norfloxacin 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 results found in this study is strongly supported by different other researchers. The study conducted by Karki16 among outpatient and inpatient of Kathmandu Medical College Teaching Hospital, the E.coli isolates were most susceptible to Nitrofurantoin. The similar study conducted by Arosio2 and Obi21 resistant to
Amoxicillin was observed. Resistance of \textit{E. coli} to Quinolones has remained rare until recently, until their use increased\cite{22}. In second generation antibiotics \textit{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 \textit{E. coli} were found to be 53.27\% (57/107), \textit{Proteus vulgaris} 100\%, \textit{Proteus mirabilis} 66.67\%, \textit{Pseudomonas aeruginosa} 83.33\%, \textit{Klebsiella pneumoniae} 44.44\%, \textit{Klebsiella oxytoca} 25\%, \textit{Enterobacter} spp 33.33\% and \textit{Citrobacter freundii} were 33.33\% MDR positive similar study done by Pokherel BM\cite{24} 64.9 percent of \textit{E. coli} were found to be MDR-strains. Higher rate of MDR was found in Kidney transplanted patients 59.09\% (13//22) than in Non kidney transplanted patients 46.41\% (71/153). However, the association of MDR and non-MDR strains in Kidney transplanted patients and Non kidney transplanted patients was found statistically insignificant (p>0.05).

5. Conclusion

Prevalence of 14.19\% of UTIs of the patients of urine showed growth of bacteria. Among 1233 urine sample, 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, \textit{E.coli} was the major isolates. Gram positive uropathogens were 5.71\% (10/175). \textit{Enterococcus} spp were predominat. There is significant difference of positive growth between male and female patients (P<0.05) and this study shows the significant association between infection among Kidney transplanted and Non-transplanted patients (p<0.05). But there was no any significant association between MDR status between Kidney-transplanted and Non- transplanted patients.

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