



**STUDY OF BACTERIAL INFECTION IN UTI AND THEIR DETERMINATION OF SUSCEPTIBILITY TO ANTIBIOTICS IN POST RENAL TRANSPLANTS**

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**ABSTRACT**

Bacterial infections are the most common pathogenic infections, during the first month of post kidney transplantation. In this study, about 164 kidney transplanted patients were assessed for the presence of the pathogenic bacteria such as *Escherichia coli*, *Klebsiella* spp. etc. Urine samples collected from the patients were cultured for the isolation of bacteria and were identified using regular biochemical tests and antibiotic resistance assays. The incidence of infection was positive for 96 (58.54%) out of 164 patients. *E. coli* was mostly isolated bacteria with a frequency rate of 32.5%, followed by *Klebsiella* spp. (20%), *Pseudomonas* spp. (11.3%), *Proteus* spp. (8.4%), *Acinetobacter* spp. (7.5%), *Citrobacter* spp. (4.3%) and *Streptococcus aureus* (12.8%). All the Gram-negative bacteria were found to be sensitive to amikacin (92.4-100%) and other isolates showed sensitive to Cefotaxime, Imepenem and Gentamicin. Thus, the present study aims to implement a new prophylaxis regimen for the treatment of post-operative infection.

**KEYWORDS:** *Bacterial infection, Post-kidney transplantation, Urinary tract infection, Antibiotic resistance*



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## INTRODUCTION

Transplantation is the last option of treatment for patients with chronic kidney failure and recurrent infection is a significant problem concerning the renal transplanted patients. Kidney transplantation possesses intense risk of developing bacterial, viral and fungal infections as a result of immunosuppressive therapy and medications<sup>1</sup>. Although, advanced surgical techniques and more effective immunosuppressive therapies are available, bacterial infections are still remains the predominant complications among the kidney transplanted individuals<sup>2</sup>. With proper pre-operative screening and immunization, post-transplant checkups and prophylaxis, the intense of infections would be lowered and reduced. Among several post-transplant infections that occurs, urinary tract infections (UTIs) becomes most common bacterial infection in post-kidney transplanted individuals. Severe UTIs not only affects the well-being of the patient, but also gives way to several infections that must be addressed in a transplant recipient. On the other hand, the emergence of new viral infections such as cytomegalovirus (CMV), BK-virus and Epstein-Barr virus (EBV) might lead to the further complications by the development of post transplantation lymphoproliferative disorders and febrile sequence<sup>3,4</sup>. Besides these, the huge problem of life-threatening infection occurs during the first six months of post-operative period, during which transplanted patients are exposed to urethral and intra-vascular catheter access and invasive instruments. Urinary tract infection associated with vascular catheterization is usually common infection in transplanted patients and it is the chief source of nosocomial infection and septicemia<sup>5</sup>. Treatment for bacterial infections becomes more complex and it depends on various factors such as gender, age, donor source, graft rejection, time on dialysis and medical environment, etc. Over the recent years, patients are exposed to a wide range of medications over specific drugs in the view of resistance to the causative microorganism in the transplanted individuals<sup>6</sup>. Several retrospective studies have been carried out worldwide showing the etiological patterns of pathological infection among transplanted recipients. However in India, the studies on bacterial infection and the pattern of antibiotic resistance are only rarely studied. Accordingly, the present investigative study targeted to record the common clinical incidence and causative agents of pathogenic infection among the renal transplanted recipients (RTR's). The occurrence of different bacterial strains isolated from infected patients and their resistance pattern against various drugs and antibiotics was also studied. This study is considered as essential for the proper prevention and treatment of post-renal transplantation infection.

## MATERIALS AND METHODS

### (i) Samplings

A total of 164 kidney transplanted patients referred by the "Nephrologists, Tertiary care centre, Chennai" were investigated in this retrospective study during the time between 2011 and 2015. The patient's age, gender and whether they received a living or deceased-related kidneys were considered for this study. Data were collected using a questionnaire regarding demographic and clinical data. There were 108 (65.85%) males and 56 (34.14%) females with an age range of 19-60 years (mean  $45.2 \pm 12.6$  years). A midstream clean catch specimen of urine was collected in a sterile container (4-5 ml) and immediately processed in a laboratory. All the transplanted recipients received cefazolin 2g pre-operative and norfloxacin 200 mg as a post-operative prophylaxis. Patients who have underwent kidney transplant from 1<sup>st</sup> March 2010 to 31<sup>st</sup> December 2014 were included. In presence of a bacterial infection, the same was excluded during analysis of the incidence of that infection in all the patients during the post-transplant period.

### (ii) Isolation and Identification of Microorganism

Using a calibrated inoculating loop, a measured amount of urine sample was taken and inoculated into NA (Nutrient Agar) medium. The plates were incubated for 24 h and observed for bacterial growth. A loop full of culture was taken and streaked onto one side in blood agar and Mac Conkey agar medium by making one vertical streak, then a cross streak at 90 degrees and incubated at 37°C for 24h. After 24 h of incubation, the number of suspected colonies on each plate was recorded. The plates with >50 colonies showing potentially significant growth were identified and then were subjected to gram staining and characterized biochemically according to Bergey's Manual of Systematic Bacteriology<sup>7</sup>.

### (iii) Antibiotic sensitivity testing

The antibiotic susceptibility testing of the well grown bacterial cultures was determined by modified Kirby-Bauer disc diffusion method as described by Bauer *et al.*<sup>2</sup> according to CLSI guidelines. The bacterial cultured medium was spread on Muller-Hinton agar plates and antibiotic discs were placed on them. To determine resistance, different antibiotics were chosen such as, Gentamicin (10 µg), Norfloxacin (10 µg), Imepenem (10 µg) Nalidixic acid (30 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Amikacin (30 µg), Cephalothin (30 µg), Cefotaxime (300 µg) and Nitrofurantoin (300 µg) (Hi-Media, India). The plates were incubated for 24 h at 37°C.

## RESULTS

Overall, 164 kidney transplanted recipients were assessed for bacterial infection. Out of 164 individuals, 96 patients were shown positive for urine culture test

(their colony count was equal or more than  $10^5$ ). Demographic and clinical information regarding the patients were summarized in Tables 1, 2 and 3, respectively. Female Renal transplant recipient (RTR) had a lower incidence of UTI (31.25%) compared to male patients (68.8%). With regard to age, 66.5% of transplants who developed UTIs were above 45 years. It can be seen in the present study that 55.7% of patients who are above 50 years developed UTIs, while the incidence was 30-45.2% for other age groups. The average age of the patients who developed UTI was  $45.2 \pm 12.6$  and those who did not develop UTI was  $31.7 \pm 16.4$  years. As seen in Table 2, 14.02% female RTRs who developed UTI received kidneys from living-related donors and 10.97% received cadaveric kidneys. On the other hand, 48.17% of the males who developed UTI received kidneys from living-related donors and 26.83% received cadaveric kidneys. Of the 164 urine samples collected, a total of 430 bacteria were isolated. Out of 430 bacteria isolated, Gram-negative bacilli accounted for 84% of the positive cultures, while Gram-positive cocci were 16%. The frequency of isolated microorganisms and their relation to sex is given in table 3. More than half of the participants developed post-transplantation UTIs (58.54%) (Table 1). *E. coli* was the most common pathogen isolated from transplanted

patients with 32.5% followed by *K. pneumonia* (20%), *P. aeruginosa* (11.3%), *P. mirabilis* (8.4%), *Acinetobacter* (7.5%), *Citrobacter* (4.3%), *S. aureus* (12.8) and *C. albicans* (3.2). In general, most of the UTIs (75.3%) were detected within the first month after the transplantation, recurrent infection was observed in 20.5% of the RTRs (28% for females and 35.6% for males) and they occurred mostly during  $21 \pm 14$  days after the transplant. The isolated bacterial showed wide differences in their susceptibility towards the tested antibiotics. The antibiotic sensitivity patterns of the bacteria isolated from the urine specimens are presented in Table 4 and Fig 1. Based on the antibiotic susceptibility test results, it is proven that all the bacteria recovered from RTRs showed a high degree of resistance to the antibiotics; amikacin and gentamicin. Most of the isolated bacteria in this study were resistant to Amikacin (92.4%), Gentamicin (80.8%), and Cefotaxime (73.8%). The Gram-negative bacilli isolated from RTRs were sensitive to gentamicin, Imepenem, Ofloxacin and Cefotaxime (85.9%- 100%), and Gram-positive cocci were showed 100% resistance. The amikacin was found to be more effective against *Pseudomonas* spp. and *Acinetobacter* spp. *Escherichia coli* was sensitive to gentamicin, Norfloxacin, Imepenem and Amikacin, (92.5%) and to Nalidixic acid, Cefotaxime (CTX) and Nitrofurantoin (86.4- 89.4%).

**Table 1**  
**Occurrence of bacterial UTI infection among renal transplanted patients**

Description	Developed UTI	Not Developed UTI	Developed UTI	Total
Gender	Male	42	66	108
	Female	26	30	56
	<b>Total</b>	<b>68</b>	<b>96</b>	<b>164</b>
Transplant Donor	Living Related	47	55	102
	Deceased	21	41	62
	<b>Total</b>	<b>68</b>	<b>96</b>	<b>164</b>
Age factor	Below 20	2	4	6
	Between 20 and 30	19	18	37
	Between 30 and 40	15	45	60
	Between 40 and 50	21	7	28
	Between 50 and 60	5	14	19
	Above 60	6	8	14
<b>Total</b>	<b>68</b>	<b>96</b>	<b>164</b>	

**Table 2**  
**Incidence of infection according to donor source**

Gender	Source of the transplanted kidney		Total
	Living-related	Deceased	
Male	79	44	123
Female	23	18	41

**Table 3**  
**Time of infection and frequency of bacteria in male and female patients**

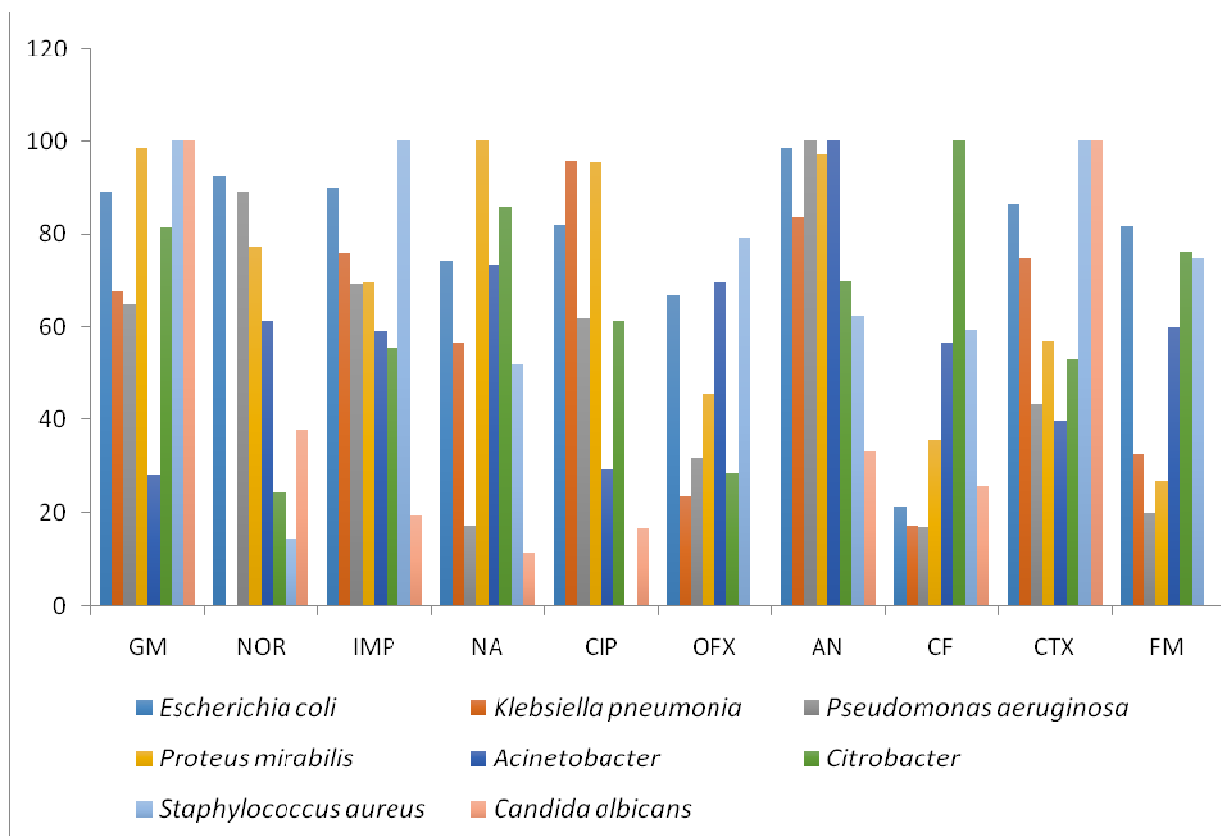
Isolated Bacteria	Male (%)	Female (%)	Percentage (%)	Time of infection	
				Below one month	Above one month
<i>Escherichia coli</i>	65.5	34.5	32.5	24	8
<i>Klebsiella pneumoniae</i>	77.7	22.3	20	16	4
<i>Pseudomonas aeruginosa</i>	45.6	54.4	11.3	10	1
<i>Proteus mirabilis</i>	61.2	38.8	8.4	6	2
<i>Acinetobacter</i>	70.4	29.6	7.5	4	3
<i>Citrobacter</i>	23	77	4.3	3	1
<i>Staphylococcus aureus</i>	59.4	40.6	12.8	4	8
<i>Candida albicans</i>	76.9	23.1	3.2	3	0

**Table 4**  
**Frequency of antimicrobial sensitivity pattern of bacteria isolated from urine specimens**

Isolates	GM	NOR	IMP	NA	CIP	OFX	AN	CF	CTX	FM
<i>Escherichia coli</i>	89.4	92.5	90.2	74.3	81.9	66.7	98.5	21.2	86.4	81.6
<i>Klebsiella pneumonia</i>	67.5	53.2	76	56.5	95.8	23.5	83.7	17.4	75	32.7
<i>Pseudomonas aeruginosa</i>	65	89.2	69.3	17.4	62	31.9	100	17	43.5	20
<i>Proteus mirabilis</i>	98.4	77.4	69.5	100	95.4	45.7	97.2	35.8	56.7	26.7
<i>Acinetobacter</i>	28.5	61.5	59.1	73.5	29.5	69.5	100	56.5	39.8	60
<i>Citrobacter</i>	81.3	24.4	55.4	85.7	61.4	28.6	69.7	100	52.9	76.3
<i>Staphylococcus aureus</i>	100	14.5	100	51.8	0.0	79.2	62.4	59.5	100	75
<i>Candida albicans</i>	100	37.8	19.4	11.4	16.7	0.0	33.3	25.6	100	0.0

Gentamicin (GM), Norfloxacin (NOR), Imepenem (IMP), Nalidixic acid (NA), Ciprofloxacin (CIP), Ofloxacin (OFX), Amikacin (AN), Cephalothin (CF), Cefotaxime (CTX) and Nitrofurantoin (FM).

**Figure.1**  
**Antimicrobial sensitivity pattern of isolated bacteria**



## DISCUSSION

Organ transplantation is considered as the standard therapy for the failure of solid organs including Liver<sup>8</sup>, heart<sup>9</sup>, lungs<sup>6</sup> and kidney<sup>5</sup>. Several advanced surgical techniques and therapies were available to improve the quality and the survival rate of the transplant recipients. The immunosuppressive therapy used after transplantation usually results in the complications including graft rejections and infections. Bacterial infections are found to be the common infections following the immunosuppressive therapy. The highest incidence of bacterial infections was occurred in kidney transplant recipients (78%) followed by lung transplant recipients (54%), liver transplant recipients (47%) and heart transplant recipients (30%)<sup>10, 11</sup>. Hence in the present investigation, the occurrence of bacterial pathogens in the urine samples of the renal transplant recipients was assessed. In a study conducted by Soemann and Hori<sup>12</sup>, they revealed that about 75% of UTIs were occurred after the kidney transplantation are within the first 3 months after surgery. The clinical data of the study patients revealed that about 44% of the transplant recipients receiving cadaveric kidneys. A study conducted by Wyner<sup>13</sup> revealed that the incidence of bacterial infection was more in case of cadaveric kidneys compared to the kidneys from living related donors. While

enumerating the pathogenic bacteria present in RTRs, *E.coli* was found to be predominant among the other pathogens. Along with enteric Gram negative bacteria, Wyner<sup>13</sup> found *P. aeruginosa*, enterococci and Staphylococci were also found to cause UTI in renal transplant recipients. In a study conducted by Dantas et al.<sup>14</sup>, *Enterobacter cloacae* was predominant among the post-transplant UTIs. Antibiotic susceptibility results revealed that the bacterial isolates were resistant towards most of the tested antibiotics, owing to their role in causing UTI in renal transplant recipients. Rivera-Sanchez et al.<sup>6</sup> have isolated Gram negative bacteria from renal transplant recipients in Mexico and most of the bacteria isolated were found to be resistant against Ciprofloxacin and Ampicillin antibiotics. Similarly, Valera et al.<sup>15</sup> also isolated beta lactamases resistant bacteria from renal transplant recipients.

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## CONFLICT OF INTEREST

This research work was not funded by any financial agencies and there is no conflict of interest.

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