Bacterial Profile of Asymptomatic Bacteriuria among Renal Transplant Recipients

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ABSTRACT

Introduction: Urinary tract infection in renal transplant recipients is often asymptomatic. Repeated screening for bacteriuria is routinely done to treat them if necessary. It is thus mandatory that antibiotic policy be framed based on the antimicrobial susceptibility testing. This cross sectional study was undertaken to determine the etiological agents of asymptomatic bacteriuria among renal transplant recipients and the antibiotic resistance pattern.

Material and Methods: Clean catch midstream urine samples of 50 asymptomatic renal allograft recipients were subjected to quantitative culture and antibiogram over a period of three months. Statistical analysis was done using SPSS software 15.0.Proportional data of the study was tested using Pearson's Chi square test.

Results: Asymptomatic bacteriuria was diagnosed in 42% of patients among whom 43.9% had living related and 33.3% deceased donor transplantations [P=0.095 (Not significant)]. Among these patients, 35.8% were females and 63.6% were males [P=0.1 (Not significant)]. The frequency of asymptomatic bacteriuria patients at various time intervals since transplant were: 0-6mths (44.4%), 7-12mths (35.2%), 13-18mths (20%) and 19-24 months (60%) [P=0.296 for prevalence at < 1 year versus > 1yr (Not significant)].

Conclusions: No statistical significance was found between asymptomatic bacteriuria, type of transplant, gender and time since transplantation. Antibiotic resistance was significantly noted among Enterobacteriaceae isolates.

Keywords: Asymptomatic Bacteriuria, Renal Allograft Recipients, Bacterial Profile

INTRODUCTION

Urinary tract infections (UTI) are the commonest bacterial infections after renal transplantation. Studies have indicated that the frequency of urinary tract infections is higher in renal transplant recipients than in the general population, with an incidence of 4-74%. As a result of immunosuppression these patients are mostly clinically. Persistent untreated asymptomatic bacteriuria (ABU) has proved to have a grave impact on allograft function and patient survival.[1-3]

Asymptomatic bacteriuria is often detected when the bacteria are isolated with a significant colony count in a patient with absence of symptoms or signs suggestive of urinary tract infection. Early detection is useful because it may be a forerunner to a symptomatic urinary tract infection.[4]

The lack of guidelines for screening of outpatients or treatment of asymptomatic bacteriuria in renal transplant recipients leads to unsystematic and unnecessary treatment with antibiotics.[5,6]

Though microbiological screening for ABU is part of a routine workup in the post transplant period, there is paucity of data in the Indian literature regarding the etiological agents and the antimicrobial susceptibility pattern. This study was conducted with the aim of determine the etiological agents of asymptomatic bacteriuria and their antimicrobial susceptibility pattern among renal transplant recipients. This study outcome would be useful to draft antibiotic policy in such patients.

MATERIAL AND METHODS

The cross sectional study was done for a period of three months. The study was approved by the Institutional Ethics Committee and patient consent obtained. Fifty asymptomatic adult patients who underwent renal transplantation in the past two years attending the outpatient transplant clinic for routine review were included in the study. Patients presenting with clinical symptoms of UTI such as lower abdominal pain, fever, dysuria, and haematuria and those currently on urinary catheterisation and haemodialysis were excluded.

Male and female patients were instructed to collect single or two consecutive clean catch midstream urine samples respectively in a screw capped wide mouthed sterile container. The sample was transported immediately to the laboratory for culture.[3] A loopful of well mixed uncentrifuged urine was placed on a clean glass slide, stained by Gram stain and examined under oil immersion objective for pus cells, epithelial cells and bacteria.

The bacteria were identified by standard biochemical reactions. The freshly collected urine was mixed well and the colony count estimated on Cystine Lactose Electrolyte Deficient Media by semi quantitative culture by the standard loop method.[18] Asymptomatic bacteriuria was diagnosed on isolation of a single organism in counts of $\geq 10^5$ cfu/mL in women in two consecutive clean-catch voided urine specimens and in men as a single clean-catch voided urine specimen.[5]

Antibacterial susceptibility testing was done on Mueller-Hinton agar (Hi-media laboratories, Mumbai). Standard strain of *Pseudomonas aeruginosa* (ATCC 27853) and *E.coli* (ATCC 25922) was used as control and tested in a similar way. The zone of inhibition was measured and interpreted according to CLSI guidelines.[19]

Isolates of Enterobacteriaceae which gave zone diameters of ≤ 27 mm to Cefotaxime in the initial screen tests were phenotypically confirmed by the double disk synergy test. Positive control

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Klebsiella pneumoniae ATCC 700603 (Hi-media) and negative control *E. coli* ATCC 25922 were used as control strains.

STATISTICAL ANALYSIS

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) software 15.0. The proportional data of this study were tested using Pearson's Chi square analysis test χ^2. Statistical significance was given if P<0.05.

RESULTS

The study population consisted of 39 males and 11 females in the age group from 13 -60 years. Renal allograft recipients from living related donor constituted 82% (n=41) of the cases and those from deceased donors 18% (n=9). 70% of study population comprised of patients who received a renal transplant 1-12 months ago.

Among the 50 patients included, 21 (42%) had asymptomatic bacteriuria (ABU). Among the 41 live related donor recipients, 18 patients had ABU (43.9%) while 3 of the 9 deceased donor renal recipients developed ABU (33.3%) [P=0.095]. [Fig 1]. ABU was detected in 14/39 (35.8%) of males and 7/11 (63.6%) of females [P=0.1]. The proportion of patients with ABU at 0-6 mths, 7-12 mths, 13 -18 mths and 19-24mths are shown in Table 1. [P=0.296 for frequency at < 1 year versus > 1yr (Not significant)].

The direct Gram stain of 15 of 21 (71.4%) patients with ABU revealed pus cells and bacteria. The remaining six samples did not reveal pus cells. Gram negative bacilli were isolated in 19 (90.5%) and gram positive cocci in 2 (9.5%) samples.On culture, the common gram negative bacterial isolates were *Escherichia coli* (33.3%), *Klebsiella pneumoniae* (23.8%), *Klebsiella oxytoca* (19.04%), *Proteus vulgaris* (9.5%), *Pseudomonas aeruginosa* (4.8%).The gram positive cocci isolates were *Enterococuus faecalis* (4.8%) and *Staphylococcus epidermidis* (4.8%). [Fig 2]

All isolates of *E.coli, Klebsiella oxytoca* and *Proteus vulgaris* and 80% of *Klebsiella pneumoniae* were resistant to Trimethoprim Sulphamethoxazole.Extended spectrum beta lactamases (ESBL) production was phenotypically confirmed in 4 /7 *E.coli* isolates (57.2%), 2/5 *Klebsiella pneumoniae* isolates (40%), 3 /4 *Klebsiella oxytoca* isolates (75%) and 1 of 2 *Proteus vulgaris* isolates (50%).Thus 10 of 18 (55.5%)of Enterobacteriaceae isolates were ESBL producers.All these isolates were sensitive to Imipenem. [Table 2].

A single isolate of *Pseudomonas aeruginosa* was grown which was multidrug resistant with susceptibility only to piperacillin tazobactam and imipenem.

Among the gram positive cocci, Methicillin sensitive *Staphylococcus epidermidis* was sensitive to all drugs except norfloxacin. The single isolate of *Enterococcus faecalis* was sensitive to Ampicillin, Nitrofurantoin, Ciprofloxacin, Tetracycline, high level gentamicin and resistant to penicillin and norfloxacin.

Of the ten patients who came for follow up, only 4 (40%) showed repeated isolation of the same growth. Direct gram stain of urine on both occasions showed pus cells and bacteria. The remaining 6 patients did not yield growth. The direct gram stain of these patients did not show pus cells on both occasions. The rest of the patients (n=11) who had ABU and pyuria could not be followed up.

Time since transplant	Asymptomatic bacteriuria	
	No.	%
0-6 months (n=18)	8	44.4%
7-12 months (n=17)	7	35.2%
13-18 months (n=10)	3	20%
19-24 months (n=5)	3	60%

Table-1: Frequency of significant bacteriuria in various time periods since transplantation

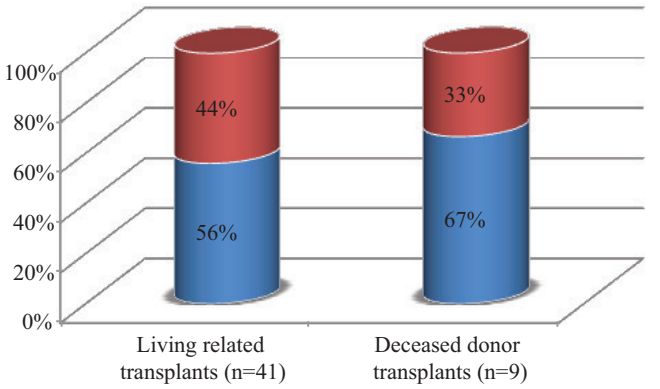

Figure-1: Prevalence of asymptomatic bacteriuria in renal allograft recipients

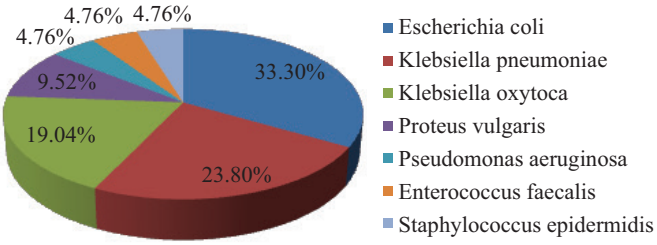

Figure-2: Etiological agents of Asymptomatic bacteriuria

DISCUSSION

Bacteremic infections are a major cause of death in kidney recipients, the urinary tract being the most frequent portal. The mortality due to bacteremia is about 11% in renal recipients.[11] The urinary tract contributes to about 73% of blood stream infection in renal transplant recipients.[13]

Asymptomatic bacteriuria (ABU) is common in renal transplant recipients, but varies widely with age, sex, type of immunosuppression and other risk factors. The incidence of ABU is reportedly twice in female transplant recipients than in males.Infections are more common in the first months after transplantation.[12] In our study 42% had asymptomatic bacteriuria with an increased occurrence in living donor renal recipients and in females. Other studies report a prevalence of 37-68%.[7,20,10] with an increased frequency of UTI in deceased donor renal recipients with a similar gender predominance[20,14]

Nearly 48% of patients with UTI are detected in the first month after transplantation. About 65% of them are asymptomatic and are usually detected on screening of urine samples.[7] Yacub et al reviewed various databases and found reports of high prevalence of ABU in first months of transplantation.[2] The observation of

Bacterial isolates	No. of susceptible isolates															
	Tmp-Sx		Gm		Nt		Cx		Nx		Ak		AS		Im	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Escherichia coli (n=7)	0	0	3	42.8%	5	71%	3	42.8%	3	42.8%	5	71.4%	7	100%	7	100%
Klebsiella pneumoniae (n=5)	1	20%	3	60%	3	60%	3	60%	5	100%	5	100%	5	100%	5	100%
Klebsiellaoxytoca (n=4)	0	0	1	25%	2	50%	1	25%	1	25%	3	75%	4	100%	4	100%
Proteus vulgaris (n=2)	0	0	1	50%	0	0	1	50%	1	50%	2	100%	2	100%	2	100%

Tmp-Sx –Trimethoprim sulphamethoxazole; Gm-Gentamicin; Nt-Nitrofurantoin; Cx-Cefotaxime; Nx-Norfloxacin; Ak-Amikacin; AS-Ampicillin sulbactam; Im-Imipenem

Table-2: Antimicrobial Susceptibility pattern of Enterobacteriaceae isolates (n=18)

our present study indicates a high frequency of ABU in the first year of transplantation.

Our study indicates that *E. coli* is the commonest etiological agent of ABU. More than 70% of UTI is reported to be contributed by Gram negative bacteria.[2] Other studies report that 61% of the pathogens in UTI after renal transplantation were *E.coli* isolates.[15] *Enterococcus faecalis* was isolated as 4.7% of the isolates in this study. A significant cause of ABU has been attributed to *Enterococcus spp* in other studies as well.[7]

The incidence of UTI and bacteremia is reported to decrease with prolonged antimicrobial prophylaxis although its role on major impact on overall graft function or survival is doubtful.[12] Trimethoprim sulphamethoxazole (TMP-SX) is the commonly used prophylactic agent. As it is well tolerated and cost beneficial and significantly reduces the incidence of bacterial infection in renal transplant recipients, especially urinary tract infections.[8] In patients who are allergic to TMP-SX, ciprofloxacin or norfloxacin are used. The onset of bacterial resistance of *E.coli* isolates to antimicrobial agents especially cotrimoxazole and cephalosporins is common after renal transplantation.[9,15,17] A wise utilisation of antibiotics is therefore mandatory.[9]

In our study none of the isolates of *E.coli, K.oxytoca, P.vulgaris* and only 20% of *K.pneumoniae* were susceptible to trimethoprim and sulfamethoxazole, which was the prophylactic antibiotic agent used in our study population.57.2% of *E.coli* isolates were resistant to norfloxacin. Senger et al reported ciprofloxacin resistance rates of 32-50% and TMP-SMX rates of 70-100% among *E.coli* isolates in various time periods after transplantation.[15]

Multidrug resistant *Pseudomonas aeruginosa* isolates and ESBL producing *E.coli* isolates cause high mortality if they cause blood stream infections in these patients.[13] In our present study 55.5% of Enterobacteriaceae isolates were ESBL producers.Parapiboon et al[12] also reported an incidence of ESBL producers of 34% among *E.coli* and *K.pneumoniae* isolates in bacteriuria in post renal transplantation cases. In our study one isolate was *Pseudomonas aeruginosa* which was multidrug resistant. As MDR *Pseudomonas aeruginosa* is significantly associated with blood -stream infection the patient was closely followed up and as the organism was isolated again, appropriate therapy was initiated.

Many authors recommend treatment of asymptomatic bacteriuria in the first months of transplantation. Some authors suggest following up the cases with asymptomatic bacteriuria, warn the patients of symptoms and treat when symptoms manifest.[16] Symptomatic UTI within one month of asymptomatic bacteriuria has been observed to be a rare event. El Amari et al studied the outcome of untreated asymptomatic bacteriuria among renal transplant recipients and found spontaneous bacterial clearance in 59% of such cases.[3] It is interesting to note that among the ten patients who came for follow-up in our study, 6 patients did not yield growth subsequently.

CONCLUSION

To conclude, asymptomatic bacteriuria was observed in 42% of renal allograft recipients. No statistical significance was found between ABU and type of transplant, sex and time of presentation. The commonest pathogens were *Escherichia coli* and *Klebsiella pneumoniae* which were notably resistant to the commonly used prophylactic agent trimethoprim sulphamethoxazole.Extended spectrum betalactamase production was detected in 55.5% of isolates. Repeated isolation of multidrug resistant bacteria requires treatment. Follow up revealed resolution of bacteriuria in 60% of cases without antibiotic treatment. The limitation of the study was a short study period due to which the impact of asymptomatic bacteriuria on graft survival was not studied.

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