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Study of Bacterial Infections Among Patients Receiving Kidney Transplant in Mashhad, Iran

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Abstract

Objectives: **Over the past 2 decades, significant advances have been made in the management of infections after transplant; however, transplant recipients are still at high risk of infectious complications. This study aimed to evaluate the prevalence of bacterial infections and antimicrobial resistance patterns in kidney transplant recipients.**

Materials and Methods: **This cross-sectional study included 356 patients who received kidney transplants, regardless of the underlying disease, from 2013 to 2015 at the Montaserieh Transplant Hospital (Mashhad, Iran). Clinical samples collected from patients were sent to the microbiology laboratory for culture processing. Typing of bacteria was conducted, and susceptibility testing was performed according to the Clinical and Laboratory Standards Institute guideline by use the of disk diffusion agar method. Data were then analyzed by SPSS software (SPSS: An IBM Company, IBM Corporation, Armonk, NY, USA) using chi-square test.**

Results: **Among 356 kidney recipients (206 men and 150 women), 115 (32.3%) received transplants from living donors and 241 (67.7%) received transplants from deceased donors. Of 356 total patients, 112 patients (31.5%) had an infection at various times after transplant. The most common gram-negative and gram-positive isolated bacteria were *Escherichia coli* and coagulase-negative *Staphylococcus*, with prevalence rates of 66.1% and 48.6%. Most of the isolates were resistant against selected antibiotics.**

Conclusions: **Because of the high prevalence of infection among transplant patients, infection prevention should receive more attention, and antibiotic susceptibility should be determined before treatment.**

Key words: *Antibiotic resistance, Coagulase-negative* Staphylococcus, Escherichia coli, *Renal transplantation*

Introduction

One of the primary and major aims in organ transplant is the effective prevention and treatment of infections. During the previous 2 decades, significant advances have been made in the management of infection after transplant. However, transplant recipients are still at high risk of infectious complications, due to the surgical interventions, immunosuppressive therapy, and exposure to the environment.[1] In transplant patients, there are generally 3 times the incidences of infections after transplant, which are usually divided as those that occur during the first month posttransplant, those that occur 2 to 6 months posttransplant, and those that occur more than 6 months posttransplant.

Most infections in the first month, especially the first 2 weeks after transplant, occur in relation to technical and operative factors. These infections include pneumonia, wound infection, those related to intravenous catheter, and urinary tract infection.[2] In addition are complications that result from infections causing the death of organ transplant recipients and in some cases organ transplant rejections, especially infections that are caused by drug-resistant bacteria.[2-4] Urinary tract infections are the most common problem after kidney transplant. Almost 70% of these infections are caused by gram-negative bacteria, especially *Escherichia coli* and *Klebsiella pneumoniae*.[4] In addition to gram-negative bacteria, some gram-positive bacteria, including

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Staphylococcus and *Enterococcus* species, are known to cause infection in these patients.[5] The primary concern in the treatment of these infections is resistance of bacteria to antimicrobial agents through different mechanisms. For example, resistance in gram-negative bacteria mostly occurs through extended-spectrum beta-lactamases (ESBLs) and carbapenemase enzymes.[4,6] In gram-positive bacteria, resistance occurs through penicillinase[7] and genes that cause resistance to vancomycin.[7,8] Therefore, the aim of this study was to evaluate the prevalence of bacterial infections, their types, and the antibiotic resistance rate in kidney recipients.

Materials and Methods

This cross-sectional study included 356 patients who received kidney transplants at the Montaserieh Transplant Hospital (Mashhad, Iran), regardless of the underlying diseases, from March 20, 2013 to March 20, 2015. Clinical samples collected from patients were sent to the microbiology laboratory for culture processing. Samples were cultured on suitable media (blood agar, MacConkey agar, and so forth) and incubated overnight. Detection and identification of isolates were done using microbiology and chemical tests such as oxidase, catalase, and growth on tryptic soy agar, SIM (sulfide-indole-motility), LIA agar, urea, and Simon citrate media. The typing of gram-negative and gram-positive bacteria was carried out, and susceptibility testing was performed according to the Clinical and Laboratory Standards Institute guideline by use of disk diffusion agar method. In brief, a bacterial suspension with concentration of 0.5 MF (MacFarland standard 1.5×10^8 cfu/mL) was prepared in brain-heart broth medium. The bacterial suspension was spread densely with sterile swab on Mueller-Hinton agar medium.

Antibiotic disks used in this study included gentamycin, amikacin, nitrofurantoin, ciprofloxacin, imipenem, chloramphenicol, meropenem, cephalexin, amoxicillin, norfloxacin, nalidixic acid, cotrimoxazole, ceftriaxone, tetracycline, ofloxacin, ceftazidime, ceftizoxime, cefixime, vancomycin, ampicillin, erythromycin, and clindamycin (High Media Company, India). Obtained results were analyzed by SPSS software (SPSS: An IBM Company, IBM Corporation, Armonk, NY, USA) using chi-square test.

Results

Of 356 patients who received kidney transplants (206 male and 150 female), 115 (32.3%) received transplants from living donors and 241 (67.7%) received transplants from brain-dead donors. Of 356 patients, 112 (31.5%; 67 females and 45 males) had positive cultures at various times after transplant. In total, 164 isolates were detected, of which 29 isolates (17.7%) were from patients who received kidneys from living donors and 135 isolates (82.3%) were from patients who received kidneys from brain-dead donors (in 22 patients, more than 1 or 2 different isolates were detected; also, 30 patients had positive cultures over 2 or 3 periods after transplant). Infection rates were 21% during the first month after transplant, 13.3% between 2 and 6 months after transplant, and 5.6% at 6 months after transplant. The 164 isolates (127 cases of gram-negative and 37 cases of gram-positive) were isolated from different sources (128 samples were from urine, 17 samples from blood, and 10 samples from discharge).

The most common gram-negative and gram-positive bacteria causing infections were *Escherichia coli* and coagulase-negative *Staphylococcus*, with prevalence rates of 66.1% and 48.6% (Table 1). Based on antibiotic susceptibility testing, the highest and lowest rates of antibiotic resistance among gram-positive bacteria were against nalidixic acid (100%) and nitrofurantoin (35.1%), respectively (Table 2). The highest and lowest rates of antibiotic resistance among gram-negative bacteria were against amoxicillin (93.4%) and meropenem (19.6%), respectively (Table 3). Unfortunately, 19 isolates (11 isolates of gram-negative and 8 isolates of gram-positive bacteria) were resistant to all antibiotics. The 11 gram-negative-resistant isolates included 4 *Klebsiella pneumoniae*, 4 *Escherichia coli*, and 3 *Acinetobacter* isolates. The 8 gram-positive-resistant isolates included 5 coagulase-negative *Staphylococci*, 2 *Enterococcus* species, and 1 *Streptococcus* species.

Table 1. Prevalence of Gram-Negative/Positive Isolates in Patients With Positive Culture

Gram Negative		Gram Positive	
Type of Bacteria	Number	Type of Bacteria	Number
Escherichia coli	84	*Coagulase-negative Staphylococci*	18
Klebsiella species	32	*Streptococcus species*	8
Acinetobacter species	6	*Enterococcus species*	6
Enterobacter species	2	*Staphylococcus aureus*	5
Proteus species	2		
Pseudomonas species	1		
Total	127	Total	32

Table 2. Results of Susceptibility Testing for Gram-Positive Isolates

Antibiotic	Coagulase-negative *Staphylococci* (18), %	*Streptococcus* species (8), %	*Enterococcus* species (6), %	*Staphylococcus aureus (5)*, %
Vancomycin				
S	22.2	87.5	66.6	100
R	77.8	12.5	33.3	0
Gentamycin				
S	16.7	25	16.6	60
R	83.3	75	83.4	40
Amikacin				
S	0	37.5	16.6	-
R	100	62.5	83.4	-
Nitrofurantoin				
S	72	37.5	50	100
R	28	62.5	50	0
Ciprofloxacin				
S	22.2	12.5	0	-
R	77.8	87.5	100	-
Chloramphenicol				
S	50	25	-	-
R	50	75	-	-
Cephalexin				
S	11	12.5	0	0
R	89	87.5	100	100
Ampicillin				
S	28	12.5	50	20
R	72	87.5	50	80
Norfloxacin				
S	28	0	0	-
R	72	100	100	-
Nalidixic acid				
S	0	0	0	-
R	100	100	100	-
Co-trimoxazole				
S	22.2	25	0	40
R	77.8	75	100	60
Ceftriaxone				
S	28	0	0	0
R	72	100	100	100
Tetracycline				
S	11	0	-	-
R	89	100	-	-
Erythromycin				
S	16.7	-	-	40
R	83.3	-	-	60
Clindamycin				
S	28	-	-	60
R	72	-	-	40

Abbreviations: R, resistance; S, susceptibility

Table 3. Results of Susceptibility Testing for Gram-Negative Isolates

Antibiotic	*Escherichia coli* (84), %	*Klebsiella* species (32), %	*Acinetobacter* species (6), %
Gentamycin			
S	38	43.7	16.6
R	62	56.3	83.3
Amikacin			
S	58.3	50	50
R	41.7	50	50
Nitrofurantoin			
S	53.5	34.4	0
R	46.5	65.6	100
Ciprofloxacin			
S	31	28.1	16.7
R	69	71.9	83.3
Imipenem			
S	66.6	59.4	33.3
R	33.3	40.6	66.6
Chloramphenicol			
S	50	84.4	-
R	50	15.6	-
Meropenem			
S	83.3	75	66.6
R	16.6	25	33.3
Cephalexin			
S	8.3	9.4	-
R	91.6	91.6	-
Amoxicillin			
S	8.3	3.1	0
R	91.6	96.9	100
Norfloxacin			
S	28.5	28.1	-
R	71.5	71.9	-
Nalidixic acid			
S	22.6	18.7	-
R	77.4	81.3	-
Co-trimoxazole			
S	12	9.4	0
R	88	90.6	100
Ceftriaxone			
S	12	9.4	16.7
R	88	90.6	83.3
Tetracycline			
S	7.1	12.5	-
R	92.9	87.5	-
Ofloxacin			
S	33.3	43.7	-
R	66.6	56.3	-
Ceftazidime			
S	17.8	12.5	0
R	82.2	87.5	100
Ceftizoxime			
S	25	18.7	-
R	75	81.3	-
Cefixime			
S	8.3	0	-
R	91.7	100	-

Abbreviations: R, resistance; S, susceptibility

Discussion

Urinary tract infection is the most common bacterial infection in kidney transplant recipients, and *Escherichia coli* is the most common bacteria that can be isolated from these infections. One of the risk factors for urinary tract infection is receiving a kidney from a donor after brain death.[9] Of 164 isolates, we had 135 cases isolated from patients who received transplants from donors after brain death, which was statistically significant (P = .01). It is noted, however, that 67.7% of transplants were from people who had brain death. Another risk factor for infection is related to female sex. In our study, of 112 patients who had a positive culture, 67 patients (59.8%) were females; it should be noted that, in our cohort of 356 transplant patients, 150 were women (42.1%) and 206 were men (56.4%). Furthermore, of the 67 positive cultures in female patients, 53 received kidneys from patients after brain death and 14 received kidneys from living donors. Of the 45 positive cultures in male patients, 37 received their grafts from donors after brain death and 8 received grafts from living donors. Other risk factors included the use of catheters and prolonged shunts and increased suppression of the immune system.[9]

One way to prevent infections after transplant is prophylaxis with antibiotics such as co-trimoxazole. In those who have allergies to these antibiotics, prophylaxis with nitrofurantoin is recommended.[10] In our study, the rate of resistance to co-trimoxazole was between 88% and 100% among the most common gram-negative bacteria causing urinary tract infections, which showed the highest resistance to these antibiotics. Use of antibiotics such as third-generation cephalosporins for prophylaxis can lead to bacterial resistance and can result in ESBLs in kidney transplant recipients.

Due to the overuse of these antibiotics, the resistance rate has increased; therefore, the drug choices for treatment of these infections are limited.[4] Extended-spectrum beta-lactamases are a class of beta-lactamase enzymes; its diagnosis is particularly important in the treatment of infections and prevalence of antibiotic resistance.[11] These enzymes cause complete hydrolysis of oxyimino beta-lactams, which are present in structures of third-generation cephalosporins.[12] The production of ESBLs in bacteria not only reduce the effectiveness of treatment with beta-lactams, especially broad-spectrum cephalosporins and monobactams, but also can lead to development of multiple resistance to other antimicrobial agents such as aminoglycosides and fluoroquinolones, which are widely used for treatment of infections caused by bacteria.[13] Extended spectrum beta-lactamases produced mostly by *Klebsiella pneumonia*e and *E. coli*, in other *Enterobacteriaceae species*, and in other nonfermented gram-negative bacteria like *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have also been identified.[14]

In our study, resistance to third-generation cephalosporins was between 75% and 100%, indicating resistance of these gram-negative bacteria to these antibiotics. The highest resistance was against cefixime (91.7% to 100%), and the lowest resistance was against ceftizoxime (75%). It should be noted that, because resistance to broad-spectrum cephalosporins occur by different mechanisms and beta-lactamase enzymes are one of them, diagnosis for this type of resistance is done using combination disks with beta-lactamase inhibitors such as clavulanic acid, tazobactam, and sulbactam,[15] where the common antibiogram methods are used in most microbiology laboratories. In our study, because of not using combined cephalosporins disks for all isolates to detect the ESBLs, it was impossible to determine the exact amount of resistance. Regarding the high level of resistance of gram-negative isolates to these antibiotics, it was expected that the prevalence of isolates producing these enzymes would be high.

For 7 isolates, resistance has been reported. In a study conducted in 2010, which included the period from 2007 to 2010, resistance increased 14%, mainly because of increased prevalence of organisms producing ESBLs.[16] This has increased concerns to select an effective antibiotic for treatment. Carbapenems (such as imipenem and meropenem) and β-lactam antibiotics are widely used to treat infections as one of the last lines of therapy. Carbapenem resistance is a major threat in treatment of nosocomial infections, which have several mechanisms, such as changes in the purines, expression of efflux pumps, and beta-lactamases hydrolyzing the carbapenems (carbapenemase).[17-19] Prevalence of carbapenem-resistant gram-negative bacteria between transplant recipients can cause increased mortality in these patients.

In our study, resistance to imipenem was significantly more than meropenem (the resistance rate to imipenem in *Klebsiella* and *E. coli* was 40.6% and 33.3%, respectively, with 25% and 16.6% for meropenem, respectively). In study by Lanini and associates, resistance rate to carbapenems was 26.5% among organ recipients. The most resistance was caused by *Klebsiella isolates*, which is consistent with our study. It is worth noting that the resistance rate in isolates of *Klebsiella species* has been shown to be 49.1%; the difference may be because most of the isolates were isolated among recipients of hearts and lungs.[6]

In addition to gram-negative bacteria resistance to cephalosporins and carbapenems, controlling gram-positive strains resistant to the spread of beta-lactam antibiotics (which are resistant to penicillinase, such as oxacillin and glycopeptide-like vancomycin), including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) isolates, are of special importance. In addition to development of resistance to these antibiotics, colonization in patients before and after transplant plays a major role in incidence of infection after transplant. In a meta-analysis conducted in 2014 by Ziakas and colleagues,[5] the MRSA colonization rate in patients was 8.5% and colonization rates of VRE

in transplant patients before and after surgery were 11.9% and 16.2%. This showed that infection after transplant in these patients was approximately 6 to 10 times higher than in other patients. Therefore, it is necessary for patients to be checked for these resistant isolates before transplant and for necessary actions to be taken to remove them.[5] In the present study, of 5 St*aphylococcus aureus* isolates, 2 cases were identified as MRSA; of 6 *Enterococcus isolates*, 2 cases were identified as VRE.

Conclusions

Regarding the high prevalence of infection in kidney recipients, first, control of infection should be performed during patient hospitalization. Second, the most effective antibiotics for prophylaxis must be selected. Third, new methods of susceptibility testing by phenotypic and possible genotypic methods should be used. Furthermore, to prevent transmission of MRSA from carriers to these susceptible patients, transplant center personnel should be evaluated for carriage of these bacteria.

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