Arrival Date: 29.09.2020 | Published Date: 22.10.2020 | 2020, Vol: 5, Issue: 8 | pp: 113-125 | Doi Number: http://dx.doi.org/10.46648/gnj.134

Frequency Of Extended Spectrum Beta Lactamase in Multiresistance Gram Negative Bacteria Isolated From Kidney Transplantation Patient

Böbrek Nakli Hastalarından İzole Edilen Çoklu Direnç Gram Negatif Bakteride Uzatılmış Spektrum Beta Laktamaz Sıklığı

Fattma A. Ali[1], Bashar Mohammed Saleh Ibrahim[2], Fatima Najmaddin Ham Muhammad[3], Solaf Abdwlrahman Jwhr[4], Zryan Tofeq Hamakhdr[5]

ABSTRACT

Infections by bacteria remain a major cause of morbidity and mortality in transplant recipients. Since the pattern of infections change continually due to evolving donor recipient characteristics, surgical techniques and immunosuppression regimens ,with widespread use of antibiotics for prevention and treatment in transplant recipients the prevalence of resistance to antibiotics among infection by bacteria is increasing. Extended spectrum beta-lactamases (ESBL) are especially common in *E. coli* and *Klebsiella* species **(**Tuğmen, etal. 2016:64**)**. This family of clinically important enzymes is transported via large plasmids, causing multiple resistance and can easily spread between strains (Saygılı, etal. 2017: 223). In this study, 300 wound and urine samples were taken from the patients who applied to Zheen international and Rizgary teaching hospital in Erbil city. Isolates were detected using microscopic, morphological and biochemical tests in January-November 2018. In addition, antibiotic susceptibility test was performed against 19 antibiotics using the Vitec 2 compact system according to the standard protocol. Isolation of 26 (8.67%) . Gram-negative bacteria from 300 samples taken from kidney transplant patients was distributed according to their sources. The highest gram-negative bacteria percentage was isolated from 17 men (65.4%) and 9 women (34.6%). The most common isolates were *E. coli* 21 (7%), *K. pneumonia* 2 (0.67%), *P. aeruginosa* 1 (0.33%) and *Enterobacter spp* 2 (0.67%). The highest resistance rate was against ampicillin, amoxicillin and ampicillin / clavulanic acid (100%). İsolates, resistance rates meropenem (92%), imipenem (88%), nalidixic acid (48%) and also recorded high resistance rate to different classes of antibiotics particularly in urine samples. In this study, it was aimed to determine gram negative bacteria among kidney transplant recipients and to investigate the antimicrobial susceptibilities of causative agents. As a result, it can be expected to lead to the development of multidrug resistance against commonly used antibiotics after transplantation and high social and economic costs. It is necessary to regularly monitor the frequency of ESBL secreting bacteria in hospitals and prevent their spread.

Keywords: kidney transplantation, Gram negative bacteria, Antibiotic resistant, ESBL

ÖZET

Bakterilerin neden olduğu enfeksiyonlar, nakil alıcılarında önemli bir morbidite ve mortalite nedeni olmaya devam etmektedir. Gelişen verici alıcı özellikleri, cerrahi teknikler ve immünosupresyon rejimleri nedeniyle enfeksiyon paterni sürekli olarak değiştiğinden, transplant alıcılarında önleme ve tedavi için antibiyotiklerin yaygın kullanımı ile bakterilerin neden olduğu enfeksiyonlar arasında antibiyotiklere direnç prevalansı artmaktadır. Genişletilmiş spektrumlu beta-laktamazlar (GSBL) özellikle *E. coli* ve *Klebsiella* türlerinde yaygındır **(**Tuğmen, vd. 2016:64). Klinik olarak önemli enzimlerden oluşan bu aile, büyük plazmitler yoluyla taşınır, çoklu dirence neden olur ve suşlar arasında kolayca yayılabilir (Saygılı, vd. 2017: 223). Bu çalışmada Erbil kentinde bulunan Zheen uluslararası ve Rizgary eğitim hastanesine başvuran hastalardan 300 yara ve idrar örneği alınmıştır. İzolatlar, Ocak-Kasım 2018'de mikroskobik, morfolojik ve biyokimyasal testler kullanılarak tespit edildi. Ayrıca standart protokole göre Vitec 2 kompakt sistemi kullanılarak 19 antibiyotiğe karşı antibiyotik duyarlılık testi yapıldı. Böbrek nakli hastalarından alınan 300 örnekten 26 (% 8.67) Gram-negatif bakteri izolasyonu kaynaklarına göre dağıtıldı. En yüksek gram-negatif bakteri yüzdesi 17 erkek (% 65,4) ve 9 kadından (% 34,6) izole edildi. En yaygın izolatlar *E. coli* 21 (% 7), *K. pneumonia* 2 (% 0.67), *P. aeruginosa* 1 (% 0.33) ve

[1] College of Health Sciences/ Hawler Medical University, Erbil/Iraq., fattma.ali@hmu.edu.krd, ORCİD: 0000-0002-5679-1767
[2] College of Pharmacy / Suleyman Demirel University, Department of Pharmaceutical Microbiology, Isparta / Turkey, bashardbo81@hotmail.com, ORCİD: 0000-0003-3086-0995
[3] College of Health Sciences/ Hawler Medical University, Erbil/Iraq.
[4] College of Health Sciences/ Hawler Medical University, Erbil/Iraq.
[5] College of Health Sciences/ Hawler Medical University, Erbil/Iraq.

Enterobacter spp 2 (% 0.67) idi. En yüksek direnç oranı ampisilin, amoksisilin ve ampisilin / klavulanik aside (% 100) karşı olmuştur. İzolatlar, direnç oranları meropenem (% 92), imipenem (% 88), nalidiksik asit (% 48) ve ayrıca özellikle idrar örneklerinde farklı sınıf antibiyotiklere karşı yüksek direnç oranı kaydetmiştir. Bu çalışmada böbrek nakli alıcılarında gram negatif bakterilerin belirlenmesi ve etken ajanların antimikrobiyal duyarlılıklarının araştırılması amaçlanmıştır. Sonuç olarak, transplantasyon sonrası yaygın olarak kullanılan antibiyotiklere karşı çoklu ilaç direncinin gelişmesine ve yüksek sosyal ve ekonomik maliyetlere yol açması beklenebilir. hastanelerde GSBL salgılayan bakterilerin sıklığının düzenli olarak izlenmesi ve yayılımının önlenmesi gerekmektedir

Anahtar kelimeler: Böbrek nakli, Gram negatif bakteriler, Antibiyotik direnci, GSBL

1. INTRODUCTİON

Although dreams of transplantation date back to the third century AD, this dream came true at the end of the 20th century. The first successful organ transplantation in humans and enabling the recipient to live for another 8 years is the kidney taken from the twin brother, and Joseph Murray performed it in 1954 **(**Ruiz,2018: 1; Diniz,et al. 2019: 1). Post-transplant recipients have a higher risk of nosocomial infection, the most common pathogens during this period are gram-negative bacilli (*Klebsiella pneumonia, Escherichea coli, Pseudomonasaeruginosa* and *Enterobactar spp*), gram-positive cocci (*Staphylococcus aureus* and *Streptococcus spp*) and also may contain anaerobic bacteria **(**Willmes, et al. 2018: 138). In organ and tissue transplants, the possibility of tissue rejection is reduced by providing effective immunosuppression, but this situation prepares the ground for opportunistic infections. In addition, the primary diseases of these patients, surgical and invasive procedures also increase the risk of infection. Since immunosuppression does not occur completely in the first month after transplantation, the risk of opportunistic infection is low. During this period, infections due to nosocomial or surgical intervention are seen. Post-transplant 1.-6. The period between months is the period when immunosuppression is most intense and life-threatening infections occur in this period. Although urinary tract infections (UTI) are the most common after kidney transplantation, pneumonia, surgical site infection and central catheter-related infections are also seen (Ak, etal.2013: 946; Mansury, etal. 2018: 283; Tanyel, etal. 2019:290). Bacteria in the urine in kidney transplant recipients when there are no symptoms of urine infection is called asymptomatic bacteriuria. Up to one in two people with a kidney transplant will develop a bacterial infection of the urine bacteriuria at some point after transplantation. Bacteriuria with symptoms like fever, chills, painful urination, abdominal pain and blood in urine is a urinary tract infection (UTI). Bacteriuria often occurs without symptoms and it is frequently treated with antibiotics with the idea this might help avoid subsequent UTI (Parasuraman- Julian 2013: 327a). The most common infection after kidney transplantation is urinary tract infections (UTI). Its prevalence is between 35% and it accounts for approximately 40% - 50% of all infectious complications (Säemann- Hörl 2008:58; Lee, etal. 2013: 733). In the literature, it is stated that the length of hospital stay and the frequency of re-hospitalization after discharge of patients with UTIs significantly increase compared to those who do not develop infections. Although urinary tract infections occur at any time after transplantation, their incidence is higher in the first three to six months (Hollyer- Ison 2018: 28; Coussement, etal. 2018:76). Antimetabolite (azathioprine ormycopheno latemofetil) based regimens that predispose to bone marrow suppression, and induction therapy with cell depleting antibodies such as antithymocyte globulin have been reported to have higher incidence of UTI (Tanyel, etal. 2019:290). Nevertheless, the earliest possible removal of the bladder catheter is generally advocated and it has been suggested that early catheter removal may lead to a drop in UTI rates. As mentioned above female renal transplant patients have a statistically significant higher incidence of UTIs compared to male transplant recipients (Khosroshahi, etal. 2006: 2060). For example in the study of 55% of the patients who were 65 years of age or older at kidney transplantation developed post-transplant UTIs compared to 30% of patients who were younger than 30 years (Chuang, etal. 2005: 232; Tenaillon, etal. 2010: 209). Between January 2000 and August 2002, 40 cases (patients older than 65 years) who underwent a kidney transplant were compared with 40 controls (under 65). Infections occurred in 32 cases (80%) but only 14 controls (32%). Also, in UTIs *E. coli* or *Enterococcus faecalis* was found more frequently in cases than controls. In a cohort of kidney transplant recipients in Brazil, the incidence of UTIs caused by ESBL-producing organisms gradually increased from 13% between first episodes of infection to 45% of patients with a third episode of UTI (Pinheiro, etal. 2010: 484; Yuan, etal.2018; 709). Several reports indicate a similar continued trend toward increased resistance in gram negative bacteria isolated from transplant patients (Shafiekhani, etal.2007; 3480). Pan resistance (PR) is defined as non susceptibility to all licensed, routinely available antibacterials. The impact of infection with MDR or PR bacteria on transplant recipient survival has become an important concern as several reports indicate significantly decreased survival of patients infected with such bacteria (Moreno, etal. 2007:2580). In several cohorts of transplant recipients dramatic increases in percentages of *Enterobacteriaceae* which are ciprofloxacin-resistant or produce ESBL or AmpC have been reported. Rates of ESBL producing *Enterobacteriaceae* ranged from 8% to 77% in these studies (Pinheiro, etal. 2010: 484). Specific risk factors for antibiotic resistance in transplant patients have not been systematically studied in large-scale multicenter analyses. General risk factors for acquisition of MDR bacteria are increasingly recognized to be shared among pathogens, and include prior antimicrobials, devices, longer length of hospital stay, and increased severity of underlying illness (Safdar - Maki 2002: 835). Use of fluroquinolones as prophylaxis for renal transplant recipients has been linked to surges in fluoroquinolone-resistant *Pseudomonas aeruginosa* (Rafat, etal. 2011: 349). Emergence of multidrug-resistant organisms, including ESBL-producing organisms or carbapenemase producing organisms, have been observed in transplant units and may be associated with a poorer prognosis (Linares, etal. 2007:2228).

2. METHODS

2.1. Sample Collection

A total of (300) samples were collected from different sources (urine, wound). After collection all bacterial isolates were subjected to a series of confirming tests. Clinical samples were collected from hospitalized patients with kidney transplantation from Rizgary hospital and Zhen international hospital in Erbil city during the period of January 2018 to November 2018 from male and female patients with the age of 10-79 years .For isolation of microorganisms, the specimen was directly inoculated on culture media; Blood culture and macConkey agar plates were incubated aerobically at 37°C for (24-48) hours. Pure colonies of isolated microorganisms were identified using morphological, biochemical tests, Species identification and antibiograms for pathogens were performed using Vitek 2 system (Duran, etal. 2017:110).

2.2. Antimicrobial Susceptibility Test By Vitek 2 System

All of the isolates used 19 (amoxicillin, ampicillin / cloxacillin, amoxicillin / clavulanic acid amikacin, azithromycin, aztreonam, ciprophlicacin, ceftriaxone, cefotaxime, piperacillin / tazobactam, trimiphacillin/ tazobactam). the correct mechanism of fingerprint and bacteria resistance antibiotics "It was made using the Vitek 2 system with recognition capability.

2.3. Phenotypic Screening For ESBL

Each isolate was tested using the VITEK 2 system with the antimicrobial susceptibility test extend AST-EXN8 card.This system was designed to perform both screening and confirmatory tests for phenotypic detection of ESBL on the same plate. The test comprises a panel of six wells containing ceftazidime 0.5 mg⁄ L, cefotaxime 0,5 mg⁄ L and cefepime 1,0 mg⁄ L, the rest of three wells were filled with same three antibiotics in combination with clavulanic acid (4,4 -10 mg⁄ L, respectively). Growth in each well was quantitatively assessed by means of an optical reader. The proportional reduction in growth in wells containing cephalosporin + clavulanate compared with those containing the cephalosporin alone was considered to be indicative of ESBL production. All phenotypic interpretations of ESBLs were reported as a positive ESBL screening result. Strains were reported as ESBL-negative whenever phenotypic interpretations other than ESBLs were proposed by the An expert system (AES) (Duran, etal. 2017:110).

3. ETHİCAL APPROVAL

The bacterial strains used in this research were extracted from clinical routine specimens, and patients were given verbal consent. This study has been accepted by the College of Health Sciences / Hawler Medical University Scientific and Research Ethics Committee.

4. STATISTICAL ANALYSIS

Statistical analysis performed using version 22 of the Social Sciences Statistical Package (SPSS) program. To evaluate categorical data, a chi-squared test was carried out .A P value of < 0.05 has been regarded as statistically significant.

5. RESULTS

5.1. Incidence Of Gram Negative Bacteria Isolated From Different Clinical Samples Of Hospitalized Patients With Kidney Transplantation

Twenty six (8.67%) gram negative bacteria isolates from 300 samples from patients with kidney transplantation distributed according to their source of isolation (Table 1 & Figure 1). Results showed that *E. coli* isolates are the most frequent encountered 21 (7%), while *K. pneumonia* isolates were 2 (0.67%), *P. aeruginosa* was 1 (0.33%), and *Enterobacter spp*. 2 (0.67%).

Table (1): Incidence of gram negative isolates from hospitalized patients with kidney transplantation

Isolated bacteria	No. of isolated positive		No. of isolated negative		Total	
	No.	%	No.	%	No.	%
E. coli	21	7	279	93	300	100
K. pneumonia	2	0.67	298	99.3	300	100
P. aeruginosa	1	0.33	299	99.67	300	100
Enterobacter spp	2	0.67	298	99.3	300	100
Total	26	8.67	274	91.3	300	100

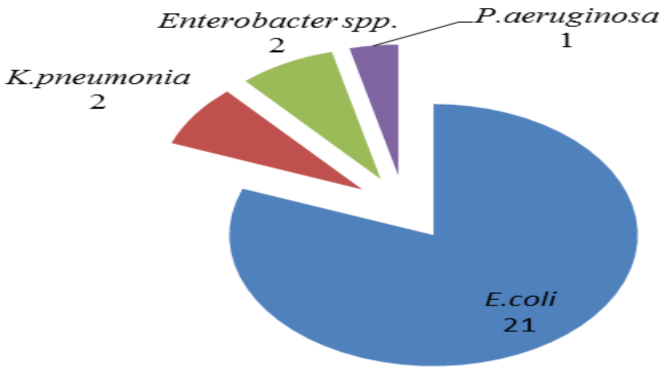

Figure (1): Incidence of Gram negative isolates from hospitalized patients with kidney transplantation

5.2. Frequency Of Gram Negative Bacteria Isolated From Different Clinical Samples From Patients With Kidney Transplantation:

Twenty six samples (8.67%) among 300 collected samples were positive. Urine was the major source of bacterial isolates collected comprising and among them 8 were positive, for E.coli 21(80%), *K. pneumoniae* 2 (7.69%), *Enterobacter spp* 2 (7.69%) and *P. auroginosa* 1(3.8%), while for wound swab is 16 samples all of them 15(93.74%) was *E.coli* and 1(3.8%) K. *pneumonia*, statistical analysis shows not significant 0.75 ($p<0.05$) relation between gram negative bacteria and different clinical sample as in table 2 and figure 2.

Table (2): Frequency of gram negative bacteria isolated from different clinical samples from patients with kidney transplantation

Isolated bacteria	Urine		Wound		Total		P value
	NO.	%	NO.	%	NO.	%	0.73
E.coli	5	19.3%	16	61.6%	21	80.9%	
K.pneumoniae	1	3.8%	1	3.8%	2	7.6%	
P.auroginosa	1	3.8%	0	0%	1	3.8%	
Enterobacter	2	7.7%	0	0%	2	7.7%	
Total	9	34.6	17	65.4	26	100%	

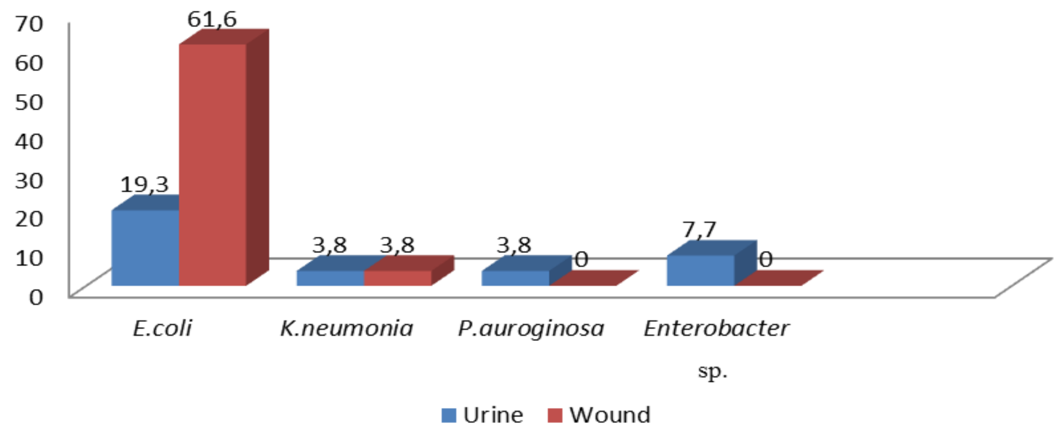

Figure (2): Frequency of gram negative bacteria isolated from different clinical samples from patients with kidney transplantation

Arrival Date: 29.09.2020 | Published Date: 22.10.2020 | 2020, Vol: 5, Issue: 8 | pp: 113-125 | Doi Number: http://dx.doi.org/10.46648/gnj.134

5.3. Relation Between Gram Negative Bacteria Species and Gender In Kidney Transplant Patients

Out of 26 (8.67%), of isolates showed higher number in male 17 (65.4%) than in female 9 (34.6%). İn male, other species showed lower number as for *Enterobacter spp*. was 2 (7.6%), *K.pneumonia* was 2 (7.6%) and *P.auroginosa* 1 (3.8%). There was no isolation in female, and it is important to notice that there was no any isolated *K.pneumonia* and *Enterobacter spp* in female, statistical analysis shows not significant 0.31 (p<0.05) correlation between gram negative bacteria and gender (Table 3 and figure 3)

Table (3): Relation between gram negative bacteria species and gender in kidney transplant patients

Isolated bacteria	Male		Female		Total		P value
	NO.	%	NO	%	NO	%	0.31
E.coli	12	46.2%	9	34.6%	21	80.8%	
K.pneumonia	2	7.7%	0	0%	2	7.7%	
P.auroginosa	1	3.8%	0	0%	1	3.8%	
Enterobacter spp.	2	7.7%	0	0%	2	7.7%	
Total	17	65.4	9	34.6	26	100%	

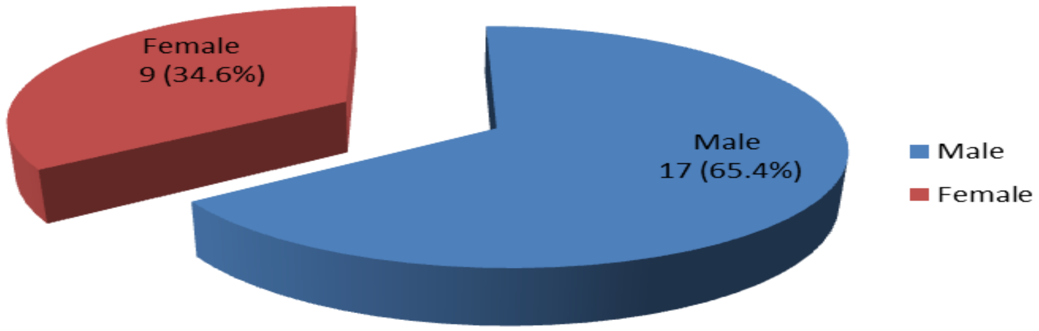

Figure (3): Relation between gram negative bacterial spiciness and gender

5.4. Distribution Of Gram Negative Bacterial Isolates According To The Age

Our result showed that out of 26 samples that were isolated from the age of 10 to 79 years, 3 (11.5%) of the gram negative bacterial isolates were from 10-19 years in which 2 of them was *E.coli* while the other was *Enterobacter spp*, 1 (3.8%) from 20-29 years which was *E.coli* while 3 (11.5%) from 30-39 years in which all of them were *E.coli* while 7 (26.9%) from 40-49 years and all of them were E.coli, and most of the bacterial isolates 8 (30.8%) isolates from 50-59 years in which 6 of them were *E.coli* and the other 2 were *K.pneumoniae* 3 (11.5%) from 60-69 years in which 1 was E.coli 1 was P.aeruginosa , 1 was Enterobacter spp and 1 (3.8%) bacterial isolate from 70-79 in which it was *E.coli* statistical analysis shows not significant 0.81 (p<0.05) correlation between gram negative bacteria and age as in table 4 and figure 4.

Table (4): Distribution of gram negative bacterial isolates according to the age

Age (year)	Types of isolates (No & %)										P value
	E.coli		*K.pneumonia*		*P.aeruginosa*		*Enterobacter spp.*		Total		
	No.	%	No.	%	No.	%	No.	%	No.	%	
10-19	2	7.69%	_	_	_	_	1	3.8%	3	11.5%	0.81
20-29	1	3.8%	_	_	_	_	_	_	1	3.8%	
30-39	3	11.5%	_	_	_	_	_	_	3	11.5%	
40-49	7	26.9%	_	_	_	_	_	_	7	26.9%	
50-59	6	23%	2	7.69%	_	_	_	_	8	30.8%	
60-69	1	3.8%	_	_	1	3.8%	1	3.8%	3	11.5%	
70-79	1	3.8%	_	_	_	_	_	_	1	3.8%	
Total	21	80.8%	2	7.69%	1	3.8%	2	7.69%	26	100%	

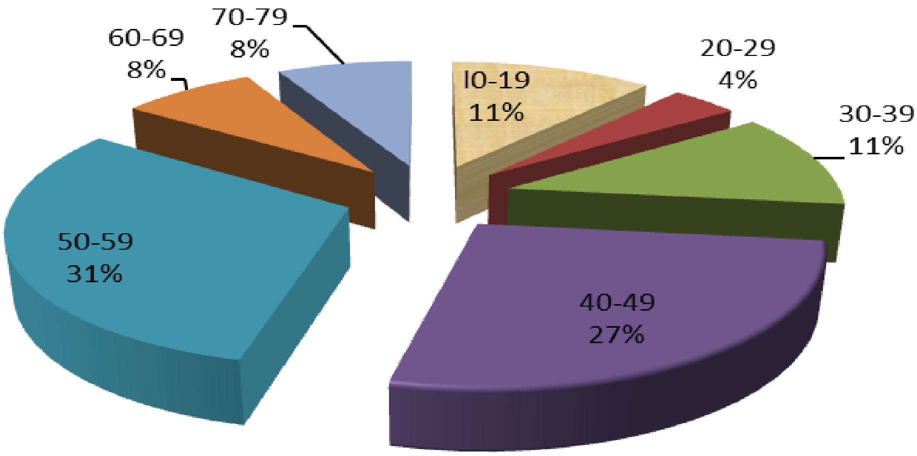

Figure (4): Distribution of gram negative bacterial isolates according to the age

5.5. Number and Percentage Of Antibiotics Resistance Among Gram Negative Bacteria

The antibiotics resistance pattern of 26 isolates of Gram negative bacteria were screened for their resistance to nineteen widely used antibiotics. All 26 Gram negative bacteria isolates showed high resistance (100%) to ampicillin, amoxicillin ampicillin/ cloxacillin, and high resistance of *E. coli* has been noticed to cefuroxime also, trimethoprim/ sulfamethoxazole, ciprofloxacin and gentamycin with different percentage rates (88%, 80%, 56%,68%), statistical analysis shows significant 0.00 ($p<0.05$) correlation between gram negative bacteria and antibiotic suseptebility as in table 5.

Table (5): Number and percentage of antibiotics resistance among gram negative bacteria

Antibiotics	Resistant		Sensitive		Intermediate		P value
	No.	%	No.	%	No.	%	
AM	25	100%	0	0%	0	0%	0.00
AMP	25	100%	0	0%	0	0%	
AMC	25	100%	0	0%	0	0%	
AK	21	84%	4	16%	0	0%	
AZM	24	96%	1	4%	0	0%	
AZN	23	92%	1	4%	1	4%	
CIP	14	56%	10	40%	1	4%	
CRO	22	88%	1	4%	2	8%	
CTX	22	88%	1	4%	2	8%	
CXM	24	96%	1	4%	0	0%	
CFM	24	96%	1	4%	0	0%	
GM	17	68%	8	32%	0	0%	
IPM	1	4%	22	88%	2	8%	
LVX	14	56%	9	36%	2	8%	
MEM	1	4%	23	92%	1	4%	
NA	24	96%	1	4%	0	0%	
F/M	7	28%	12	48%	6	24%	
TZP	12	48%	10	40%	3	12%	
TMP	20	80%	5	20%	0	0%	

AM; amoxicillin, AMP: ampicillin/cloxacillin, AMC; amoxicillin /clavulanic acid, AK; amikacin, AZM; azithromicin, AZN: Aztreonam, CIP: ciprofloxacin, CRO; ceftriaxone, CTX; cefotaxime, CXM; cefuroxime, CFM; cefixime, GM; gentamycin, IPM; imipenem, LVX; levofloxacine, MEM; meropenem, NA; nalidixic acid, F/M; nitrofurantoin, TZP; piperacillin/tazobactam, TMP; trimethoprim/sulfamethazone

5.6. Antibiotic Sensitivity In *Pseudomonas aeruginosa*

Its non-fermenter because of that we use different antibiotic sensitivity discs and it shows only resistance to(cefazoline) and sensitive to (gentamicine, ciprofloxacin, imipeneme) as shown in table (6) and figure 5.

Table (6): Antibiotic sensitivity in *Pseudomonas aeruginosa*

Antibiotics	Sensitive	%
Cefepime	S	
Cefazoline	_	R
Ceftazidime	S	_
Ciprofloxacin	S	_
Gentamicin	S	_
Imipenem	S	_
Levofloxacin	S	_
Piperacillin/Tazobactam	S	_
Tobramycin	S	_
Total	8	1

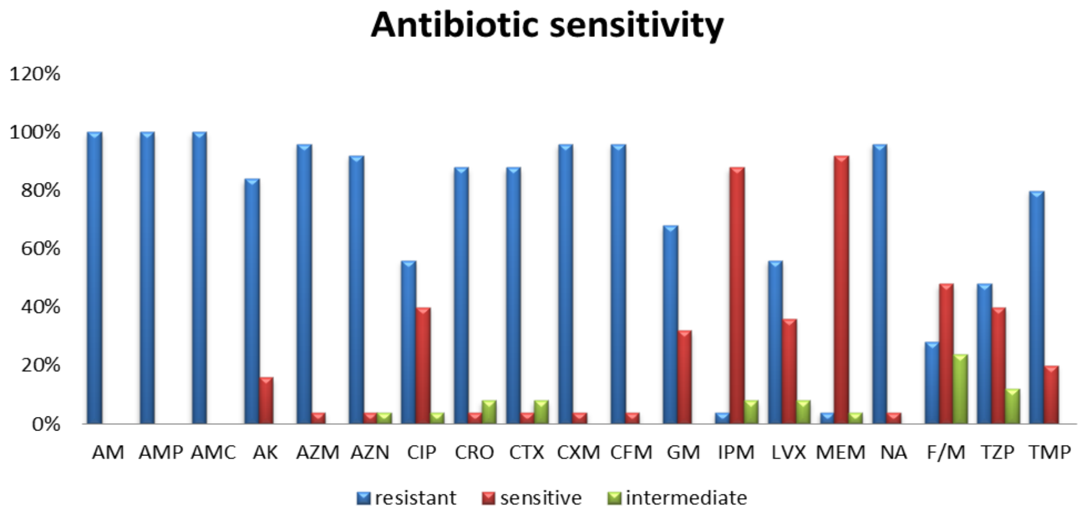

Figure (5): Number and percentage of antibiotics resistance among gram negative bacteria

5.7. Resistant Rate Of Antibiotics Among Gram Negative Bacterial Isolates

Our results showed that most isolates of Gram negative bacteria showed high resistance rate against the most of the antibiotics, on the other hand *E.coli* , *K. pneumoniae* and *Enterobacter spp*. Showed 100% resistance against ampicillin, amoxicillin and cloxacillin, statistical analysis shows not significant 0.55 ($p<0.05$)correlation between gram negative bacteria and antibiotic resistances as in table 7.

Table(7): Number and percentage of antibiotics resistance among gram negative bacteria

Antibiotics	***E.coli* : N=21**		***K.pneumoniae:* N=2**		***Enterobacter* spp. N=2**		**Total: N=25**		**P value**
	No.	%	No.	%	No.	%	No.	%	
AM	21	100%	2	100%	2	100%	25	100%	0.55
AMP	21	100%	2	100%	2	100%	25	100%	
AMC	21	100%	2	100%	2	100%	25	100%	
AK	17	80.1%	2	100%	2	100%	21	84%	
AZM	21	100%	1	50%	2	100%	24	96%	
AZN	20	95.2%	2	100%	1	50%	23	92%	
CIP	11	52.3%	2	100%	1	50%	14	56%	
CRO	19	90.4%	2	100%	1	50%	22	88%	
CTX	19	90.4%	2	100%	1	50%	22	88%	
CXM	21	100%	2	100%	1	50%	24	96%	
CFM	21	100%	2	100%	1	50%	24	96%	
GM	15	71.4%	1	50%	1	50%	17	68%	
IPM	0	0%	1	50%	0	0%	1	4%	
LVX	4	19.0%	2	100%	1	50%	7	68%	
MEM	0	0%	1	50%	0	0%	1	4%	
NA	21	100%	2	100%	1	50%	24	96%	
F/M	4	19.0%	1	50%	2	100%	7	28%	
TZP	10	47.6%	1	50%	1	50%	12	48%	
TMP	18	85.7%	2	100%	0	0%	20	80%	

5.8. Frequency Of Antibiotic Resistance In Different Clinical Samples

Our Gram negative bacterial isolates showed high resistant rate to different classes of antibiotics multidrug resistance (MDR) particularly in urine samples, in which there is 100% resistant rate against 14 antibioyics (ampicillin, amocicillin, cloxacillin, amoxicillin/clavulanic acid, amikacin, azithromicin, aztreonam, ciproflixacin, ceftriaxone, cefotaxime, cefuroxime, cefixime, gentamycin, levofloxacine, nalidixic acid, trimethoprim/sulfamethazone) statistical analysis shows not significant 0.99 ($p<0.05$) correlation between antibiotic resistance and clinical sample as shown in table (8).

Table (8): Frequency of antibiotic resistance in different clinical samples

Antibiotics	Urine		Wound		Total		P value
	NO.	%	NO.	%	NO.	%	
AM	7	100%	18	100%	25	100%	0.99
AMP	7	100%	18	100%	25	100%	
AMC	7	100%	18	100%	25	100%	
AK	7	100%	14	77.7%	21	84%	
AZM	7	100%	17	94.4%	24	96%	
AZN	7	100%	16	88.8%	23	92%	
CIP	7	100%	7	38.8%	14	56%	
CRO	7	100%	15	83.3%	22	88%	
CTX	7	100%	15	83.3%	22	88%	
CXM	7	100%	17	94.4%	24	96%	
CFM	7	100%	17	94.4%	24	96%	
GM	7	100%	10	55.5%	17	68%	
IPM	0	0%	1	5.5%	1	4%	
LVX	7	100%	7	88.8%	14	56%	
MEM	0	0%	1	5.5%	1	4%	
NA	7	100%	17	94.4%	24	96%	
F/M	2	28.5%	5	27.7%	7	28%	
TZP	4	57.1%	8	44.4%	12	48%	

5.9. Frequency Of ESBL Producing Gram-Negative Bacteria Isolates

Vitek2 system revealed that out of 26 gram-negative isolates included in the study,17(65.4%) isolates were ESBL producer. These ESBL producing bacteria belonged to 2 different species, out of the 4 species isolated from different clinical samples of kidney transplant pateint. ESBL production was very common among *E.coli*, where out of the 21(80.7%) E.coli isolates, 16(76.1%) were ESBL producer. However, within *K.pneumonia* isolates, 1 out of 2(50%) isolates were ESBL producers, representing 50%. For *P.aeroginosa* and *Enterobacter* were not ESBL producer at all among our isolated samples. statistical analysis shows not significant 0.08($p<0.05$) correlation between gram negative bacteria isolated and ESB as in table (9) and figure 6.

Table (9): Frequency of ESBL producing gram-negative

Isolated pathogen	ESBL positive		ESBL negative		Total	
	No.	%	No.	%	No.	%
E.coli	16	76.2%	5	23.8%	21	80.7%
K.pneumonia	1	50%	1	50%	2	7.69%
P.aeruginosa	0	0%	1	100%	1	3.85%
Enterobacter	0	0%	2	100%	2	7.69%
Total	17	65.4%	9	34.6%	26	100%

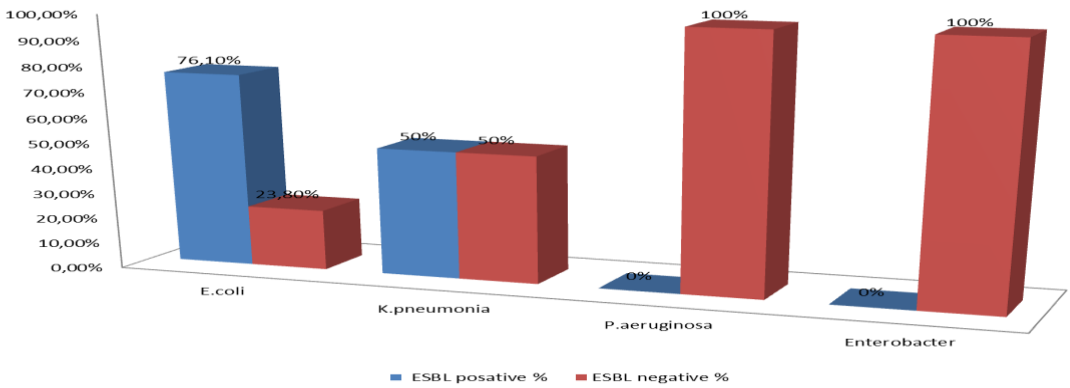

Figure (6): Frequency of ESBL producing Gram-negative bacteria isolates

6. DISCUSSION

Kidney transplantation is the best treatment for patients at the end-stage of renal disease (Ganji, etal. 2015: 397). Number of kidney recipients is estimated more than 1.4 million worldwide, with 8% rising incident rate, annually (Ghonemy, etal. 2016: 113). İn our study, Out of 300 samples that were subjected to Rizgary and Zheen international hospital in Erbil city during the period of January 2018 to November 2018, 26 (8.67%) were positive for *Enterobacereacea* and *P.aeruginosa*. All isolated bacterias were identified by using microscopical, morphological, biochemical tests and Vitek 2 compact system. In a study conducted from June 2010 to January 2011, (Adnan,etal. 2015:1) 50 of 150 samples from kidney transplant patients were positive for gram-negative bacteria. Compared to our sample and positive cases, the number was much higher. In another kidney transplant study, 127 of 356 patients were positive for gram-negative bacterial isolates. Also, even though the number was not detectable between the total sample numbers, the most common gram-negative isolates were *E.coli* 21 (7%) followed by *K.pneumoneae* 2 (0.67%) and *Enterobacter spp* 2 (0.67%), *P. aeruginosa* It was 1 (0.33%) **(**Mansury, etal. 2018: 283). *E.coli* which we encountered with a rate of 7% in 21 of 300 cases in our study, was determined similar to other studies. However, the differences in numbers and in isolated gram negative organisms compared with some studies may be due to the total size of the sample, may be due to the conditions in which the operation was performed or the degree of aseptic techniques taken. The hospitals in which the patient stayed after surgery or the environmental conditions, or the antibiotic profiles taken by pre- and post-operative patients, these conditions vary from one hospital to another or from one city to another, or in cross-country results leading to differences (Adnan,etal. 2015:1). Among the 26 positive samples included in our study, we found, the highest number of isolates in 17 (65.4%) wound samples 9 (34.6%) in urine samples.In a study conducted, they found 128 of 164 positive cases from urine and 10 from wound **(**Mansury, etal. 2018: 283). these results do not agree with our results. These isolated organisms are often associated with certain and serious illnesses, where urinary tract infections are the most common problem after kidney transplant. Almost 70% of these infections are caused by gram-negative bacteria, especially *E. coli* and *K. pneumoniae* (Ramadas, etal. 2014: 1), and bacteremia is considered a potential cause of sepsis and affects graft results (Yuan, etal.2018; 709). In a study of Medicare claims for 28942 renal transplant recipients, the cumulative incidence UTI during the first 6 months post-transplant was 17% in both men and women. However, by three years post-transplant, there was a significantly higher incidence in women (60%) compared to men (47%), p< 0.001 (Abbot, etal. 2004: 353 ; Adnan,etal. 2015:1). In our study, The number of isolates are higher in male samples 17 (65.4%) than in female samples 9 (34.6%). Differences in the number of gram-negative isolates in males and females may be due to the larger sample size in males than females (Pinheiro, etal. 2010: 484; Ganji, etal. 2015: 397), because our target populations are related only to patients randomly admitted to the hospital. Infections remain a major cause of morbidity and mortality in transplant recipients. Since the patter of infections change continually due to evolving donor–recipient characteristics, surgical techniques and immunosuppression regimens. Infections account for 16% of patient deaths and 7.7% (14% in patients >65 years of age) of death censored graft failure in kidney transplant (KT) recipients (Parasuraman- Julian 2013: 327a). Our results showed that the prevalence of gram-negative bacteria (30.8%), was found in kidney transplant patients aged 50-59 years, suggesting that the elderly are at higher risk of hospital infections than younger recipients. One of the current limitations with the available clinical evidence base is a significant proportion of clinical trials (16.0%) excluded kidney transplant recipients over 65 years of age. This leaves a gap in the literature with regards to the optimal immunosuppressant regimen among the elderly. Adaptive and innate immunity decreases with age, and older kidney allograft recipients may not require the same level of immunosuppression as given per standard. This is especially important in the context of age being a strong risk factor for both malignancy and infection after kidney transplantation (Asra, etal. 2014:832). Our finding showed that gram negative bacteria remarkable variable to their resistance to antibiotic use, negative bacteria we collected in Erbil city, All gram negative bacteria we isolated showed (92%) sensitivity to cefepime and meropenem. In a study, trimethoprim, ciprofloxacin and gentamicin revealed the highest percentage of resistance (80%, 56%, 68%), respectively. This study showed meroprnem to be the most effective antibiotic against all gram-negative bacilli, whereas amoxicillin and ampicillin were the least effective antibiotics. All patients were given prophylactic antibiotics immediately after surgery. In the study, the high resistance to antibiotics showed that the infection could occur over sufficiently long periods after kidney transplantation and was not caused by catheter use (Salim- Jwaij 2020: 2568). High resistance to amikacin and ciprofloxacin was reported in the same study. they also agree with our study, but this has been shown to agree to some extent with the current result, with the most effective antibiotic being ciprofloxacin, in contrast to our study where meropenem was the most effective antibiotic. This is predicted to be due to the treatment regimen in those countries and, on the other hand, the use of standard antibiotic susceptibility testing technique by researchers, on the one hand, and the abuse of antibiotics. In the two different hospitals in Erbil city used antibiotics ampicillin and amoxicillin all bacterial isolated are 100% resistance to both antibiotics. The high rate of resistance to ampicillin and amoxicillin may reflect the fact that these are the most commonly subscribed antibiotics in hospitals and also the most easily available in the community without prescription and because they are very cheap in terms of cost and so subject to abuse. General increment of infections in kidney transplant patients over the last decade. This trend is shift from cardiovascular disorders to infectious diseases as the main cause of death; many reports indicated admission rates enhancement for infections complications during the 1st year post-transplant (Gołębiewska, etal. 2011: 2985; Lofaro, etal. 2015:1). Our result showed that there was high resistant rate among gram negative bacterial isolates especially to common antibiotics like amoxicillin, ampicillin/ cloxacillin, amoxicillin/clavulanic acid in which all isolates of *E coli* 21(100%), *K pneumoniae* 2(100%), *Enterobacter spp* 2(100%) were resistant to these antibiotics. Also E.coli was totally resistant to azithromicin 21(100%), cefuroxime 21(100%), cefixime 21(100%) and nalidixic acid 21(100%), and highly resistant to aztreonam 20(95%), ceftriaxone and cefotaxime 19 (90.4%), it show total sensitivity to imipenem and meropenem 0 (0%). *K.pneumoniae* showed higher resistant in which it was totally 2 (100%) resistant to amikacin, aztreonam, ciprofixacin, cefuriaxone, cefotaxime, cefuroxime, cefixime, levofloaxacine, nalidixic acid, and trimethoprim/sulfamethazone, *Enterobacter spp*. was totally 2 (100%) resistant to amikacin, aztreonam and nitrofurantoin additionally to the 3 common antibiotics, and it was totally sensitive to imipenem, meropenem and trimethoprim/sulfamethazone 0 (0%) UTI is one of the causes of pyelonephritis, bacteremia, CMV infections graft loss, and patient survivals rate reduction (Pourmand, etal. 2007:305). Pourmand's study in Tehran in 2007 reported *Klebsiella* as the most found pathogen. UTI rate was 41.5% in their study, UTI prevalence was lower in our study 8(30.7%) the predominant causative agent was *E coli* in our search, A recent study reported UTI alone predicted an increased first-year mortality of 41% in kidney recipients (Pourmand, etal. 2007:305; Naik, etal. 2016: 241). In our study Surgical Site Infection after renal transplantation (SSIs) were diagnosed in 69.2(18/26). Emergence of multi-drug resistant organisms, including ESBL-producing organisms or carbapenemase producing organisms, have been observed in transplant units (Linares, etal. 2007:2228). One of the most important resistant mechanisms in gram-negative bacteria against betalactam antibiotics is induced by production of beta-lactamase enzymes. The new brought-spectrum antibiotics such as cephalosporins used in treatment of bacterial infections has lead to the production of a new class of brought spectrum enzymes called beta-lactamase (Yazdi, etal. 2012: 2454). This part of study was performed to assess the effect of types of beta-lactamase production on the bacteria isolated from different sources. The result represents ESBL production occurred in 17(65.4%) out of 26 gram-negative bacteria isolates with highest incidence in *E.coli* (80.7%) and *K.pneumoniae* (50%), there was not any ESBL positive among for *P.aeruginosa* and *Enterobacter* isolated samples. Our result were compared with other result and found that higher than study recorded by Vidal in the Spanish registry study, 26% of the 118 cases of symptomatic *E.coli* UTI were caused by ESBL producing organisms (Vidal, etal. 2012: 595; Goldman, etal. 2019: 141). Our result supports the evidence that *E. coli* yielded the highest percentage of ESBL producers and all isolated of *E.coli* from all urine samples were generally resistance to the ampicillin and amoxicillin. *E.coli* now became the more common in multidrug ressistance isolate and the problem of beta lactamases is now more prevalent in *Enterobacteriaceae*. In kidney transplant recipients, ESBL-producing *Enterobacteriaceae* were found to be associated with recurrent urinary tract infection (UTI); the incidence of ESBL producing *Enterobacteriaceae* increased from 13%, 38% to 45% for first, second, and third UTI episodes, respectively. The prevalence of drug resistance varies considerably by region and country (Kamath, etal. 2006: 145; Parasuraman- Julian 2013: 330b).

7. CONCLUŞİONS

Kidney transplantation is the most successful method in the treatment of end stage renal failure. However, UTI poses a major problem in such patients. In the follow-up of patients with kidney transplantation, care should be taken in terms of urinary system infections in the first year, especially in the first 120 days. Transplant patients who are hospitalized for various reasons, especially if they have a previous history of urinary tract infection, should be evaluated in terms of disease development.

Conflict of interest and financial statement : There is no conflict of interest in this study and no financial support has been received.

8. REFERENCES

Abbot KC,Swanson SJ,Richter ER,Bohen EM,Agodoa LY, Peters TG ,Barbour G, Lipnick R, Cruess DF .Late urinary tract infection after renal transplantation in the United States. Amirikan journal of kidney disease.2004.44: 353-362.

Adnan SG, Khaled AM, Abdul Baki AR, Hassan AS and Naelash AA. Urinary tract infection among renal transplant recipients in Yemen. Plos One. 2015. 10(12):1-10.

Ak O, Yildirim M, Kucuk HF, Gencer S, Demir T. Infections in Renal Transplant Patients: Risk Factors and Infectious Agents, 2013; 45(3): 944-984.

Asra K , danilea F ,James C, Sophia M , Irena B, Daniel R et al. Recipient Age and Risk for Mortality After Kidney Transplantation in England .transplantation .2014. 97(8): 832-838.

Chuang P, Parikh CR, Langone A. Urinary tract infections after renal transplantation a retrospective review at two US transplant centers. Clin Transplant. 2005.19:230–235.

Coussement J, Scemla A, Abramowicz D, Nagler EV and Webster AC. Antibiotics for asymptomatic bacteriuria in kidney transplant recipients. Cochrane Database of Systematic Reviews .2018.11357(2):10-1002.

Diniz G, Tügmen C, Sert İ. Organ Transplantation in the Turkey and the World. Tepecik Eğit. ve Araşt. Hast. Dergisi 2019;29(1):1-10.

Duran MH, Lopez-Jacome LE, Colın-Castro CA, Ceron-Gonzalez G, etal. Comparison of the MicroScan WalkAway and VITEK 2 Compact systems for the identification and susceptibility of clinical Gram-positive and Gram-negative bacteria. CENIAQ, 2017; 6(3): pp 105-114.

Ganji MR, Alatab S, Naderi GH, Ghiasi B. Association of brain-dead donor's urine neutrophil gelatinase-associated lipocalin levels with kidney allograft function. Iran J Kidney Dis. 2015;9 :394–398.

Ghonemy TA, Farag SE, Soliman SA, El-okely A, El-hendy Y. Epidemiology and risk factors of chronic kidney disease in the El-Sharkia Governorate, Egypt. Saudi J Kidney Dis Transpl. 2016.27:111–117.

Goldman JD, Julian K. Urinary tract infections in solid organ transplant recipients: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clinical Transplantation. 2019;33: 1-16.

Gołębiewska J, Dębska-Ślizień A, Komarnicka J, Samet A, Rutkowski B. Urinary tract infections in renal transplant recipients. Transplant Proc. 2011.43:2985–29990.

Hollyer I, Ison MG. The challenge of urinary tract infections in renal transplant recipients. Transpl Infect Dis, 2018;20:e12828.

Kamath NS, John GT, Neelakantan N, Kirubakaran MG, etal. Acute graft pyelonephritis following renal transplantation. Transpl Infect Dis, 2006; 8: 140–147.

Khosroshahi HT, Mogaddam AN, Shoja MM. Efficacy of high-dose trimethoprim-sulfamethoxazol prophylaxis on early urinary tract infection after renal transplantation. Transplant Proc .2006.38:2062–2064.

Lee JR, Bang H, Dadhania D, Hartono C, et al. Independent risk factors for urinary tract infection and for subsequent bacteremia or acute cellular rejection: a single center report of 1166 kidney allograft recipients. Transplantation Journal 2013; 96: 732-738.

Linares L, Cervera C, and Cofan F. Epidemiology and outcomes of multiple antibiotic-resistant bacterial infection in renal transplantation. Transplant Proc .2007. 39: 2222–2224.

Lofaro D, Vogelzang JL, van Stralen KJ, Jager KJ, etal. Infection-related hospitalizations over 30 years of follow-up in patients starting renal replacement therapy at pediatric age. Pediatr Nephrol. 2015.31:1.7.

Mansury D, Khaledi A, Ghazvini K,Sobbagh M, et al .Study of Bacterial Infections Among Patients Receiving kidney Transplant , Iran . Experimental and clinical Transplantation, 2018 .3:282-286.

Moreno A, Cervera C, and Gavalda J. Bloodstream infections among transplant recipients: Results of a nationwide surveillance in Spain. Am J Transplant. 2007.7: 2579–2586.

Naik AS, Dharnidharka VR, and Schnitzler MA. Clinical and economic consequences of first-year urinary tract infections, sepsis and pneumonia in contemporary kidney transplantation practice. Transpl Int. 2016. 9(2):241–252.

Parasuraman R, Julian K, Urinary Tract Infections in Solid Organ Transplantation. American Journal of Transplantation 2013; 13: 327–336.

Pinheiro HS, Mituiassu AM, Carminatti M, Braga AM, and Bastos MG. Urinary tract infection caused by extended-spectrum betalactamase- producing bacteria in kidney transplant patients. Transplant Proc .2010. 42: 486–487.

Pourmand G, Salem S, Mehrsai A, Taherimahmoudi M, etal. Infectious complications after kidney transplantation: A single-center experience. Transpl Infect Dis. 2007; 9:302–309.

Rafat C, Vimont S, and Ancel PY. Ofloxacin. New applications for the prevention of urinary tract infections in renal graft recipients. Transpl Infect Dis .2011. 13: 344–352.

Ramadas P, Rajendran PP and Krishnan P. Extended-spectrum beta-lactamase producing bacteria related urinary tract infection in renal transplant recipients and effect on allograft function. PLoS One. 2014; 9(3):e91289.

Ruiz,P. (2018). Transplantation pathology. Cambridge University: 2. Edition: Chapter 1: p.1-24. United Kingdom.

Säemann M, Hörl W.H. Urinary tract infection in renal transplant recipients. European Journal of Clinical Investigation, 2008; 38 (S2): 58–65.

Safdar N and Maki DG. The commonality of risk factors for nosocomial colonization and infection with antimicrobial-resistant *Staphylococcus aureus*, enterococcus, gram-negative bacilli, *Clostridium difficile*, and Candida. Ann Intern Med. 2002. 136:834–844.

Salim N, Tuwaij S. Molecular Profile of Aminoglycoside, Fluoroquinolone, and Class 1 Integron Genes among Gentamicin-Resistant Escherichia coli in Najaf City, Iraq. ,Int.J.Res.Pharm.Sci.,2020,11(2),2558-2567.

Saygılı Penkin T, Akgüneş A. Extended spectrum beta-lactamase production and antibiotic resistance rates in of Escherichia coli and Klebsiella spp. isolated from hospitalized patients: data of 2011-2015. Turk Hij Den Biyol Derg, 2017; 74(3): 221- 228.

Shafiekhani M, Mirjalili M, Vazin A. Prevalence, Risk Factors And Treatment Of The Most Common Gram-Negative Bacterial Infections In Liver Transplant Recipients: A Review.Infection and Drug Resistance 2019:12 3485-3495.

Tanyel E, Ulusoy T, a Dilek M, Yakupoğlu Y. Investigation of infections after renal transplantation. Ortadogu Medical Journal, 2019; 11(3): 288-293.

Tenaillon O, Skurnik D, Picard B, Denamur E. "The population genetics of commensal *Escherichia coli*. Nature Reviews.Microbiology. 2010.8(3):207–217.

Tuğmen C, Sert İ, Tatar BG, Kebapçı E, et al. Donor derived Pseudomonas infection in cadaver donor kidney transplantation. Tepecik Eğit. ve Araşt. Hast. Derg, 2016; 26(1):63-68.

Urinary Tract Infections in Solid Organ Transplantatio

Urinary Tract Infections in Solid Organ Transplantation

Urinary Tract Infections in Solid Organ Transplantation

Urinary Tract Infections in Solid Organ Transplantation

Vidal E, Torre-Cisneros J, Blanes M. Bacterial urinary tract infection after solid organ transplantation in the RESITRA cohort. Transplant Infect Dis. 2012.14: 595-603.

Willmes D, Coche E, Villalobos R, Kanaan N. Bacterial pneumoneae in Kidny transplant recipients . American Journal of Transplantation. 2018. 137:89-94.

Yazdi M, Nazemi A, Mirinargasi M, Jafarpour M, etal. Genotypic versus Phenotypic methods to detect Extended-Spectrum Beta- lactamases (ESBLs) in Uropathogenic *Escherichia coli*. Annuals of Biological Research;2012; (5): 2454-2458.

Yuan S, liu T,wu D ,wan O. Epidemiology, susceptibility, and risk factors for acquisition of MDR/XDR Gram-negative bacteria among kidney transplant recipients with urinary tract infections. Infection and drug reisstance. 2018 .11:707-715.