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FREQUENCY OF EXTEND SPECTRUM BETA LACTAMASE IN MULTIRESISTANCE *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIA* ISOLATED FROM KIDNEY TRANSPLANTATION PATIENTS

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ABSTRACT

In transplant recipients, infections are fatal and they can cause considerable morbidity due to constant changes of infection patterns, evolving donor–recipient characteristics, surgical techniques and immunosuppression regimens. The prevalence of resistance to antibiotics among bacteria causes infection in kidney transplant patients (KTPs). This type of infection is caused by a widespread use of antibiotics to prevent and treat infection in those patients. Objectives: The aim of the present study was to determine the incidence of urinary track infections (UTIs) among renal transplant recipients. The study also investigates the antimicrobial susceptibility pattern of causative agents. Materials and Methods: In this study, 200 samples of wound and urine were collected from patients admitted to Zheen international hospital in Erbil city in one year (January of 2019 to January of 2020). Two isolated gram negative (GN) strains were identified by using cultural, morphological, characteristics, and biochemical tests and Vitek 2 system. Results: From the 200 samples, 9 (4.5%) *E.coli and K.pneumonia* bacteria isolates were taken. Out of 9 (4.5%) of isolates, 4 isolated *E.coli* species were equal in males and females 4 (44.4%). *K.pneumonia* was 1 (11.1%) in females, and there was no isolation in males. Our results showed that urine was the major source of bacterial isolates collected comprising 6 were positive. *E.coli* 5(55.6%) for *K. pneumoniae* was 1(11.1%), while for wound swab was 3 samples *E.coli* 3(33.3%) and no *K.pneumonia*. Our results showed that most isolates of *E.coli and K.pneumoniae* were highly resistant against the majority of antibiotics, *Escherichia coli* and *K. pneumonia* showed 100% resistance against Ampicillin, Amoxicillin and Cloxacillin. The percentage of the resistance of *E. coli* to Trimethoprim/ Sulfamethoxazole, Azithromycin was high (88.9%). Also, sensitivity was as follows, Amikacin (88.89%), Ertapenem (88.89%), Gentamycin (77.78%), Meropenem (88.89%), Imipenem (88.89%), Tazobactam (77.78%). Extended-spectrum beta-lactamase ESBL production was very common among *Ecoli*, where out of the 8 (88.9%) *E.coli* isolates, 4 (44.4%) were producers of ESBL. However, *K.pneumonia* isolates were not. Conclusions: ESBL producing bacteria increase the rate of morbidity and mortality. This increase entails high healthcare costs. Also, MDR pathogens caused infections are a therapeutic challenge for clinicians and a risk for immuno-compromised patients. There are some antibiotics for the treatment of these infections. These antibiotics can be recommended mostly for non-oral means of administration but have higher risks of side effects.

Keywords: kidney transplantation, *E. coli*, *K.pneumonia*, UTI, wound infection, antibiotic resistant, ESBL.

Introduction

Infections in renal transplant (RT) people compose 26 % of the hospitalization days per year and 40 % of the all mortalities (Kee *et al*., 2004). Recently, invasive bacterial infections continue to be leading causes of mortality and morbidity in solid organ transplant people and high costs of transplantation. In Iran, renal transplantation rate is higher than around 24 cases every million people recently (Hassan zadeh *et al*., 2010). Following kidney transplantation, NG organisms cause 50–90% of UTIs. In this cases, in urine cultures, the most common isolated microorganism is *Escherichia coli*; this is also true in general population. However, there is a difference in aetiology between primary and late periods following RT (Golebiewska *et al*., 2014). Next to the transplantation of kidney, UTI is the most frequent infection (Adamska *et al*., 2015). It commonly causes failure of allograft, sepsis and mortality following transplantation. When kidneys are transplanted, one of highest risks is UTI in the first transplantation year about 60% (Veroux *et al*., 2008). There are many more risk factors such as advanced age, being female, vesicoureteral reflux and diabetes mellitus (Chuang and Parikh, 2005). Despite that UTI is most frequent bacterial infections in children, its diagnosis is often deferred because of the vague clinical outcomes. In pediatric patients, acute pyelonephritis could cause high morbidity and long-term complications such as chronic renal failure, hypertension, and renal scarring (Taneja *et al*., 2010). Also, in KTPs, *Klebsiella* species virulence factors could develop upper UTIs (Golbiewska *et al*., 2014). Furthermore, in a unit where UTI reoccurs following kidney transplant about 72% and *Klebsiella* species prevail as nosocomial infections, uropathogen and multidrug-resistant bacteria which seem to be an independent predictive factor (Silva *et al*., 2013).

When kidneys are transplanted, antibiotic are increasingly administered to patients because of the treatment of pneumocystis pneumonia (PCP), asymptomatic bacteriuria (ASB) and prophylaxis (Pilmis *et al.*, 2015).

UTI is the most frequent bacterial infections in KTPs, composing higher morbidity, enhanced costs, and high consumed medical resource. In kidney recipients (KRs), it is about 7% to 43%, which is significantly bigger than that in the general population. Post transplantation, the first year is a highest risk period (Pelle *et al*., 2007). However, recurrent UTI is common among those patients causing multidrug resistance (Bodro *et al*., 2015)

Urologic complications are the most common surgical complication after renal transplantation, causing significant morbidity and mortality. Recently, the incidence of urologic complications after renal transplantation has decreased from 12.5% to 2.5% (Emiroglu *et al*., 2016). In pyelonephritis (PN) cases, there is no often pain because the transplanted kidney is not innervated. Few transplant physicians consider all forms of UTIs occurring in RT recipients as complicated UTIs because of the altered anatomy of the UT and the immuno-compromised status (Parasuraman *et al*., 2013). The modification of risk is also a significant step to prevent UTI. A later RCT shows that UTI can be reduced when early ureteral stent removal at one week is compared with the routine stent removal at four weeks (Liu *et al*., 2017). Bloodstream infection is common in KTRs and could cause death. It is about 0.7 to 11 episodes per 100 patients a year. The percentage of septicemia mortality was 63 in the 1960s and stayed high at 24.3 even in the 21th century (Silva *et al*., 2010).

Treatments of KTRs who have sepsis become more difficult because those patients are susceptible to to kidney injury and drug interactions that immunosuppressive factors such as cyclosporin and tacrolimus cause. In addition, pathogens with different resistance to cephalosporins and fluoroquinolones and a high pervasiveness of ESBL-producing strains complicate this condition. KTRs need immunosuppressive therapies for preventing the rejection of the transplanted organ (Lim *et al*.,2013). The rising incidence of resistant strains may be due to high antibiotic exposure. Antibiotic exposure could be identified as a risk factor for the resistance in GN bacteria different circumstances (Hu *et al*., 2016). The antibiotic exposure after kidney transplantation is elevated due to treatment of asymptomatic bacteriuria (ASB) and pneumocystis pneumonia (PCP) prophylaxis.

Acquiring large plasmids is a major contributing factor for encoding resistance for multiple drug types. The recent literature shows that organisms such as *E. coli* and the *Klebsiella* with more diverse integrons and transposons are used in a multiplicity of transferable plasmids. These plasmids are able to encode every beta-lactamase type. In the mid-1980s, the description is extended to ESBL producing organisms as a universal problem (Bradford,2001). These organisms have been found continentally (Paterson *et al*., 2003). Despite the fact that ESBLs have existed in a varied range of GN bacteria, *Klebsiella pneumoniae* is the most common species for the production of ESBLs (Bradford, 2001).

Materials and Methods

Isolation and identification of *E.coli and K.pneumonia*

This study was performed in the Zheen international hospital hospitals in Erbil city from (July 2019 – January 2020). A total of 200 various samples (urine, wound) were collected from hospitalized KTPs from patients of both genders with a variety of ages and inoculated onto Blood Agar and MacConkey Agar plates. Plates were incubated at 37°C for 24-48 hours under aerobic conditions. Provisional identifications of isolated bacteria were done on the basis of Microscopic Properties, cultural characteristics and biochemical tests definitive Identification by Vitek 2 Compact. Colorimetric technology was used to identify bacterial and GN card was also utilized. According to the manufactures instructions (Biomerieux, France), the following steps are performed. In plane test tube, three ml of normal saline were put and inoculated by a lope which is filled with isolated colony. Into the Dens Check machine, the tube of the test was inserted to standardize the colony to McFarland standard solution (1.5X10 8cfu/ml). Then, into the cassette, the standardized inoculum was put followed by using a barcode to insert an identification number of the sample into the software. Then, from the barcode placed on the card during manufacture, the Vitek 2 card was read. This card was linked to the identification port of the sample. When the card was filled, the cassette was inserted in the filter module. The cassette was later moved to the reader/ incubator module. In all the next steps, instruments were used for controlling the incubation temperature and the optical card reading. For the analysis, these instruments were also used to constantly monitor and transfer test data to the computer. As the test cycle finished, the card ejection into the waste bin automatically happened.

Antimicrobial susceptibility test and ESBl by Vitek 2 system

Antimicrobial sensitivity testing and ESBL of the isolates was determined by using Vitek 2compact system, 15 an automated ID and susceptibility (AST) system (bioMe´rieux,USA). This system has an Advanced Expert System (AES) and a high sensitivity with quick and reliable specificity values (94-100%) for routine laboratory tests widely used for identifying and/or analyzing susceptibility by the use of different (AST) cards based on the expected pathogens. The card inoculation and incubation took place in the machine according to the manufacturer's instructions. The susceptibility test of Vitek 2 results were gotten as MIC values. The results can be susceptible, intermediate or resistant in accordance to the National Committee for Clinical Laboratory Standard's breakpoint Final (Clinical and Laboratory Standards Institute, 2011). The AES (software version VT2- R05.04) was utilized to interpret the results. The test was repeated the AES recommends (Eigner *et al*., 2005)

Statistical analyses

Statistical Package for Social Sciences (SPSS) software (SPSS- 19) was used for statistical analyses. Fischer exact test or 2 test was used for the analysis of the categorical data. $P<0.05$ was considered statistically significant.

Ethical statement

The research projects were cleared by the Institutional Ethics Committee of the College of Health Sciences/Hawler Medical University

Results

Nine samples (4.5%) of *Escherichia coli* and *K. pneumoniae isolated* from 200 LTPs divided on the basis of their isolation sources. The findings revealed that *E. coli* isolates were 8 (4%), while *K. pneumoniae* isolates was 1 (0.5%) as shown in table (1).

Table 1 : Incidence of *E coli and Klebseilla pneumoniae* isolates from hospitalized patients with kidney transplantation:

Isolated bacteria	No. of isolated positive		No. of isolated negative		Total	
	No.	%	No.	%	No.	%
E. coli	8	4 %	192	96 %	200	100%
K. pneumonia	1	0.5 %	199	99.5%	200	100%
Total	9	4.5 %	191	95.5 %	200	100%

Frequency of *E coli* and *K pneumoniae* isolated in different clinical samples

Nine samples (4.5%) from the whole samples were positive. Urine was the major source of bacterial isolates collected and 6 were positive. For urine, *E.coli* and K *pneumoniae* were 5 (55.56%) and (11.11%), respectively while wound swab 3 samples, *E.coli* was 3 (33.33%) with no *K. pneumoniae.* Statistical analysis showed non-significant 0.45 (p>0.05) relation between *Escherichia coli* and *K. pneumoniae* and different clinical sample as in table (2) and figure (1).

Table 2 : Frequency of *E coli and Klebseilla pneumoniae* isolated from different clinical samples from KTPs:

Isolated bacteria	Urine		Wound		Total		P value
	NO.	%	NO.	%	NO.	%	0.45
E.coli	5	55.56%	3	33.33%	8	88.89%	
K. pneumoniae	1	11.11%	0	0%	1	11.11%	
Total	6	66.66	3	33.33	9	100%	

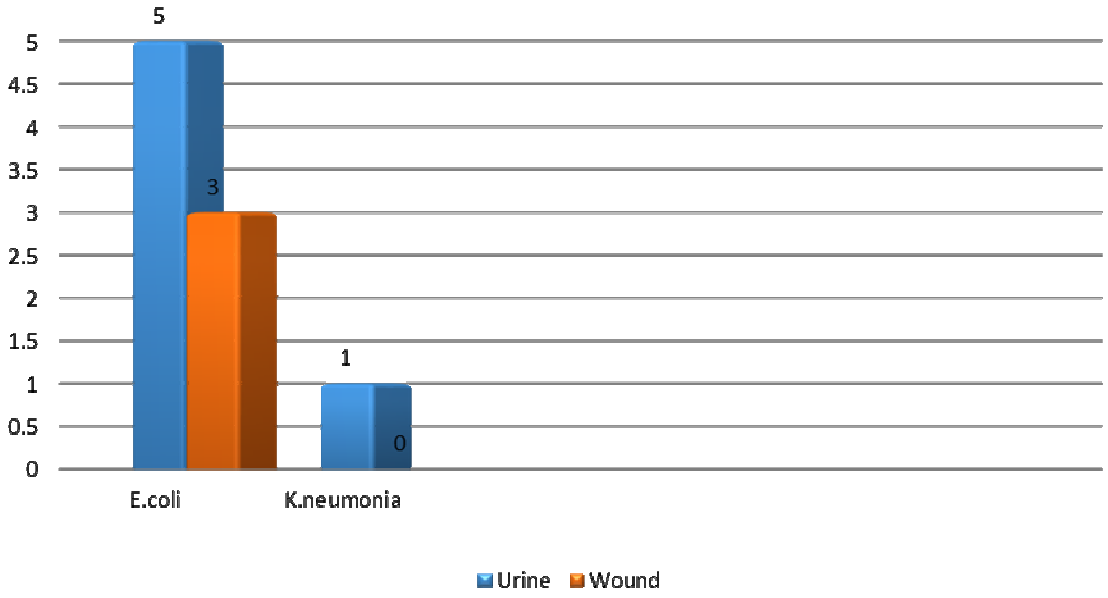

Fig. 1 : Frequency of *E. coli and K pneumoniae* isolated from different clinical samples from KTPs

Relation between *E. coli and K pneumoniae* species and gender in kidney transplant patients:

Out of 9 (4.5%) of isolates, 4 isolated *E.coli* species showed equal number in male 4 (44.44%) and female 4 (44.44%), *K.pneumoniae* was 1 (11.11%) in female. Also, there was no isolation in male and the statistical analysis showed non-significant 0.34 (p>0.05) correlation between *E coli* and *K. pneumoniae* and gender as in table (3) and figures (2 and 3):

Table 3 : Relation between *E coli and K pneumonia* species and gender in KTPs:

Isolated bacteria	Male		Female		Total		P value
	NO.	%	NO	%	NO	%	
E.coli	4	44.44%	4	44.44%	8	88.89%	0.34
K.pneumoniae	0	0%	1	11.11%	1	11.11%	
Total	4	44.44	5	55.56	9	100%	

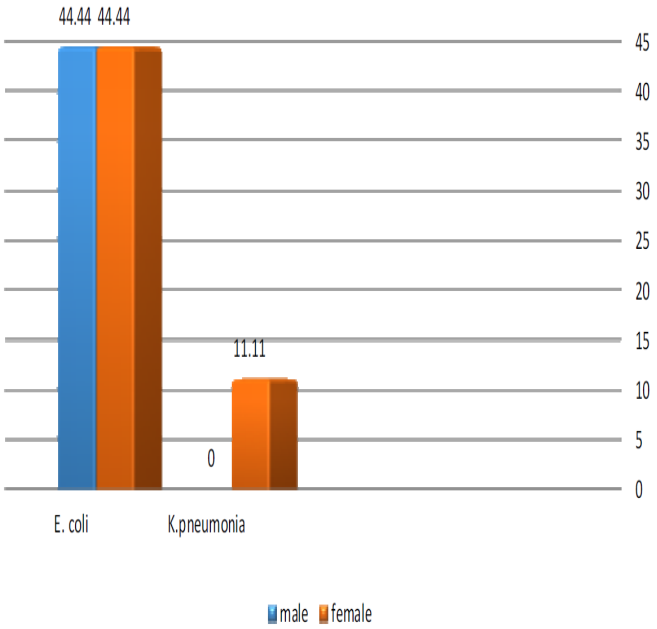

Fig. 2 : Relation between *E coli and Ka pneumoniae* and gender in KTPs

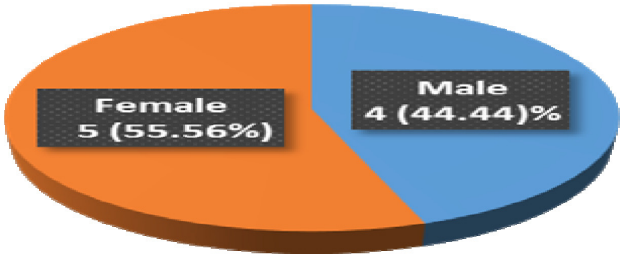

Fig. 3 : Relation between *Es coli and K pneumoniae* and gender in kidney transplant patients

Distributing *E coli and K pneumoniae* isolates from KTPS according to the age:

Our result showed that out of 9 samples that were isolated from the age of 10 to 89 years. From age 10-19, 3 (33.33%) of the *E.coli* isolates were with no cases were from 20-29 years, while 1(11.11%) *E.coli* was from 30-39 and 1 (11.11%) from 40-49 years. In addition, *E.coli* 1(11.11%) isolates were from 50-59 years and there were no *K.pneumoniae* cases from 60-69 years. Furthermore, 1 (11.11%) *E.coli* bacterial isolate was from 70-79 and 2 (22.22%) from 80-89. With no statistically significant 0.11 ($p>0.05$) correlation between *E coli* and *K pneumoniae* and age.as in table (4).

Table 4 : Distributing *E coli and K pneumoniae* isolates from KTPs according to age:

Age (year)	Types of isolates (No & %)						P value
	E.coli		*K.pneumoniae*		Total		0.11
	No.	%	No.	%	No.	%	
10-19	3	33.33%	_	_	3	33.33%	
20-29	_	_	_	_	_	_	
30-39	1	11,11%	_	_	1	11.11%	
40-49	1	11.11%	_	_	1	11.11%	
50-59	_	_	1	11.11%	1	11.11%	
60-69	_	_	_	_	_	_	
70-79	1	11.11%	_	_	1	11.11%	
80-89	2	22.22%	_	_	2	22.22%	
Total	8	88.89%	1	11.11%	9	100%	

Antibiotics sensitivity patterns among *E coli and K pneumoniae* in kidney transplantation patients:

The antibiotics resistance patterns of 9 isolates of *Ecoli and K pneumoniae* were screened for nineteen widely used antibiotics. Both bacteria isolates showed high resistance (100%) to Ampicillin, Amoxicillin, Ampicillin/ cloxacillin, followed by 88.89% resistance to Trimethoprim/ Sulfamethoxazole and Azithromycin. The statistical analysis showed a significant correlation 0.000003 ($p<0.05$) between *E coli* and *K pneumoniae* and type of antibiotic susceptibility as in table (5) and figure (4).

Table 5 : Antibiotics sensitivity patterns among *E coli and K pneumoniae* in KTPs:

Antibiotics	Resistant		Sensitive		Intermediate		P value
	No.	%	No.	%	No.	%	
Ampicilin	9	100%	0	0%	0	0%	0.000003
Ampicilin/ cloxacilin	9	100%	0	0%	0	0%	
Amoxicillin	9	100%	0	0%	0	0%	
Amoxicillin/ calv.acid	6	66.67%	3	33.33%	0	0%	
Amikacin (AK)	1	11.11%	8	88.89%	0	0%	
Gentamycin (GM)	2	22.22%	7	77.78%	0	0%	
Ciprofloxacin (Cip)	5	55.56%	4	44.44%	0	0%	
Levofloxacin	5	55.56%	4	44.44%	0	0%	
Tazobactum/Pipracilin	1	11.11%	7	77.78%	1	11.11%	
Imipenem(IPM)	0	0%	8	88.89%	1	11.11%	
Ertapenem	1	11.11%	8	88.89%	0	0%	

Meropenam	1	11.11%	8	88.89%	0		0%
Nitrofurontoin	3	33.33%	5	55.56%	1	11.11%	
Ceftriaxon (CRO)	4	44.44%	5	55.56%	0		0%
Cefotaxime (CTX)	4	44.44%	5	55.56%	0	0%	
Trimethoprime/Sulphamethazaxole	8	88.89%	1	11.11%	0		0%
Cefexime (CFM)	4	44.44%	5	55.56%	0	0%	
Aztreonam	4	44.44%	5	55.56%	0		0%
Azithromycin	8	88.89%	1	11.11%	0	0%	

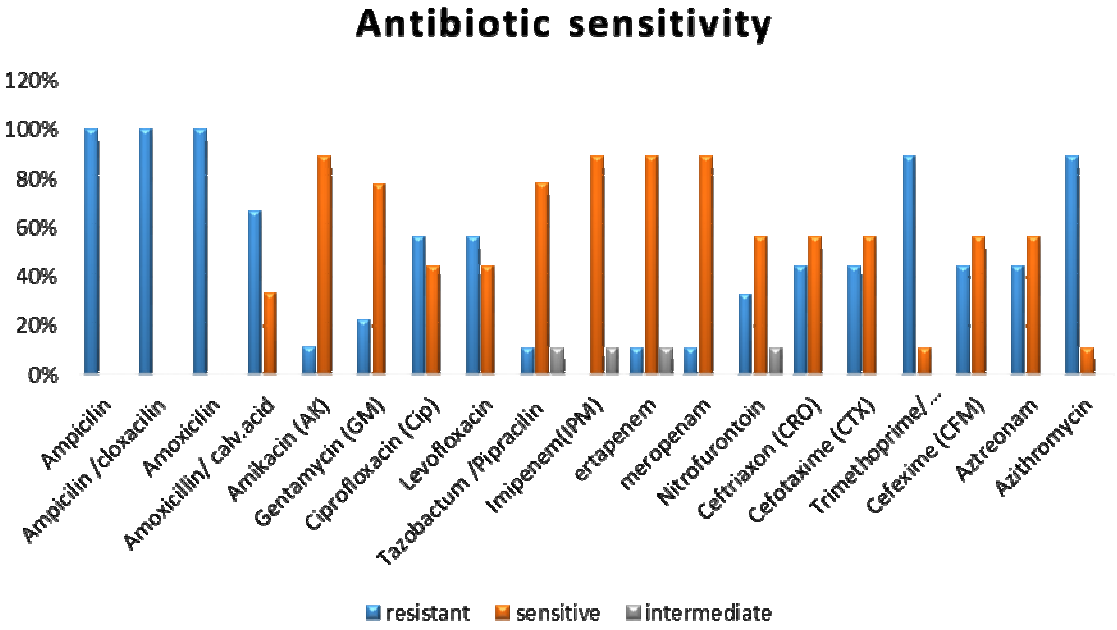

Fig. 4 : Number and percentage of antibiotics resistance among *E coli and K pneumoniae* in KTP

Resistant rate of antibiotics among *E coli and Klebseilla pneumoniae* isolates in KTP:

According to our study results, the rate of resistance of *E. coli and K. pneumoniae* against most antibiotics was high. On the other hand, *E.coli* and *K. pneumoniae* were highly resistant against Ampicillin, Ampicillin and Cloxacillin Amoxicillin as out of 9 isolates, 8 isolates showed resistance against Trimethoprime/ Sulphamethazaxole and Azithromycin. Moreover, most isolates were multiresistance to more than three class of antibiotics. In the statistical analysis, there was no significant correlation 0.98 (p>0.05) between *E coli* and *Kpneumoniae* and antibiotic resistances as in table 6.

Table 6 : Number and percentage of antibiotics resistance among *E coli and K pneumoniae* KTPs:

Antibiotics	***E.coli***		***K. pneumoniae***		**Total**		**P value**
	No	**%**	**No.**	**%**	**No.**	**%**	**0.98**
Ampicillin	8	88.89%	1	11.11%	9	100%	
Ampicillin/ Cloxacillin	8	88.89%	1	11.11%	9	100%	
Amoxicillin	8	88.89%	1	11.11%	9	100%	
Amoxicillin/calv.acid	6	66.67%	0	0%	6	66.67%	
Amikacin (AK)	1	11.1%	0	0%	1	11.11%	
Gentamycin(GM)	2	22.22%	0	0%	2	22.22%	
Ciprofloxacin(Cip)	5	55.56%	0	0%	5	55.56%	
Levofloxacin	5	55.56%	0	0%	5	55.56%	
Tazobactum/Pipracilin	1	11.11%	0	0%	1	11.11%	
Imipenem(IPM)	0	0%	0	0%	0	0%	
Ertapenem	1	11.11%	0	0%	1	11.11%	
Meropenam	1	11.11%	0	0%	1	11.11%	
Nitrofurontoin	2	22.22%	1	11.11%	3	33.33%	
Ceftriaxon(CRO)	4	44.44%	0	0%	4	44.44%	
Cefotaxime(ctx)	4	44.44%	0	0%	4	44.44%	
Trimethoprime/Sulphamethazaxole	7	77.78%	1	11.11%	8	88.89%	
Cefexime(CFM)	4	44.44%	0	0%	4	44.44%	
Aztreonam	4	44.44%	0	0%	4	44.44%	
Azithromycin	7	77.78%	1	11.11%	8	88.89%	

Frequency of antibiotic resistance in different clinical samples isolated from patients with kidney transplantation

E.coli and K. pneumoniae isolates were greatly resistant to different antibiotics particularly in urine samples, in which there is 100% resistant rate against Ampicillin, Amoxicillin, Ampicillin/Cloxacillin,. The statistical analysis revealed non-significant correlation 0.85 (p>0.05) between antibiotic resistance and clinical sample as shown in table (7).

Table 7 : Frequency of antibiotic resistance in different clinical samples isolated from KTPs:

Antibiotics	Wound		Urine		Total		P value
	No.	%	No.	%	No.	%	
Ampicillin	3	33.33%	6	66.67%	9	100%	0.85
Ampicillin/Cloxacillin	3	33.33%	6	66.67%	9	100%	
Amoxicillin	3	33.33%	6	66.67%	9	100%	
Amoxicillin/calv.acid	1	11.11%	5	55.56%	6	66.67%	
Amikacin (AK)	0	0%	1	11.11%	1	11.11%	
Gentamycin(GM)	1	11.11%	1	11.11%	2	22.22%	
Ciprofloxacin(Cip)	1	11.11%	4	44.44%	5	55.56%	
Levofloxacin	1	11.11%	4	44.44%	5	55.56%	
Tazobactum/Pipracilin	0	0%	1	11.11%	1	11.11%	
Imipenem(IPM)	0	0%	0	0%	0	0%	
Ertapenem	0	0%	1	11.11%	1	11.11%	
Meropenam	0	0%	1	11.11%	1	11.11%	
Nitrofurontoin	0	0%	3	33.33%	3	33.33%	
Ceftriaxon(CRO)	0	0%	4	44.44%	4	44.44%	
Cefotaxime(ctx)	0	0%	4	44.44%	4	44.44%	
Trimethoprime/Sulphamethazaxole	3	33.33%	5	55.56%	8	88.89%	
Cefexime(CFM)	0	0%	4	44.44%	4	44.44%	
Aztreonam	0	0%	4	44.44%	4	44.44%	
Azithromycin	3	33.33%	5	55.56%	8	88.89%	

Frequency of ESBL producing *Escherichia coli and Klebseilla pneumoniae* in kidney transplant patients

Result of Vitek2 system about ESBL revealed that out of 9 *E. coli and K. pneumoniae* isolates in the study, only 6 (66.66%) isolates were ESBL producer. Bacteria were *E. coli* isolated. ESBL production was very common among *E.coli*, where out of the 8 (88.89%) *E.coli* isolates, 6 (66.66%) were ESBL producer. However, *K. pneumoniae* isolates, in our case, were not ESBL producers with non-significant correlation 0.13 (p>0.05) between *E coli* and *K pneumoniae* isolated and ESBL producing as in table (8) and figure (5).

Table 8 : Frequency of ESBL producing *E coli and Klebseilla pneumoniae* isolates from KTP:

Isolated pathogen	ESBL positive		ESBL negative		Total	
	No.	%	No.	%	No.	%
E.coli	6	75 %	2	25 %	8	88,89%
K.pneumoniae	0	0%	1	100%	1	11.11%
Total	6	66.67 %	3	33.33 %	9	100%

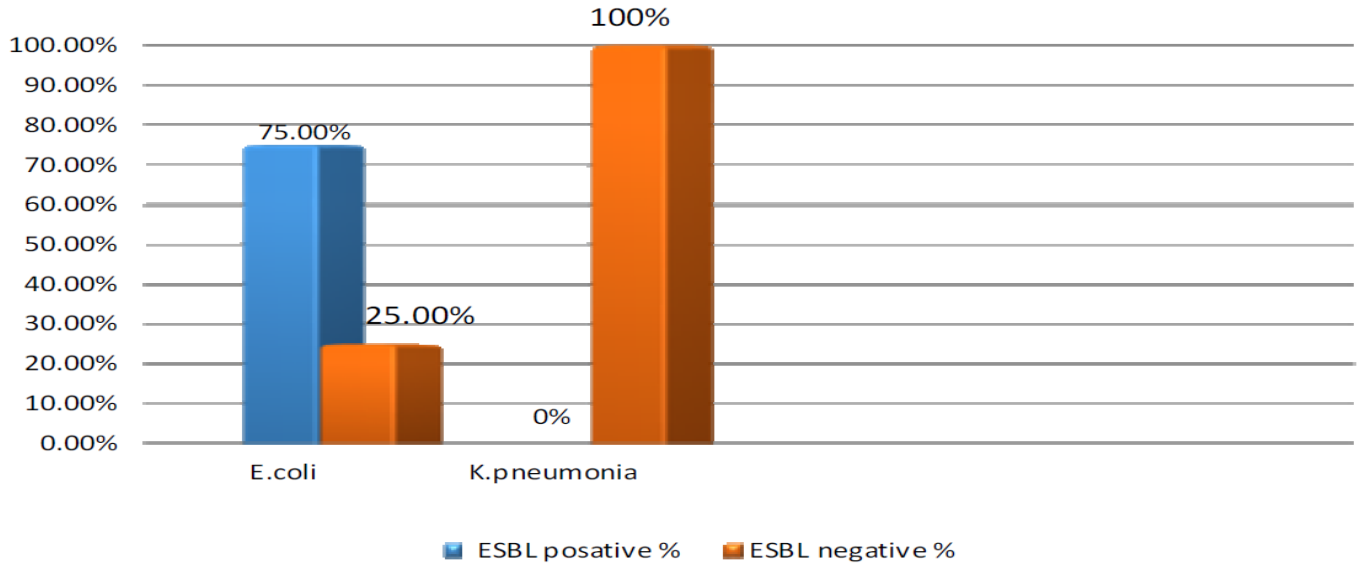

Fig. 5 : Frequency of ESBL producing *E coli and Klebseilla pneumoniae* isolates from KTPs

Discussion

The transplantation of renal is an ideal treatment for end-stage renal patients. Despite the great advances, transplantation of organ and immunosuppression for renal transplantation post-transplantation and UTI are still a major cause of morbidity and mortality (Saemann *et al*., 2008). A sample of 200 hospitalized patients with kidney transplantation was collected in 2019. Urine and wounds from those patients were collected. After final confirmation using Vitek 2 system, only 9 were confirmed as *E.coli* and *K. pneumoneai* in Zheen International Hospital in Erbil city January 2019 to February 2020. The results revealed that 9 (4.5%) were positive for *E coli and K. pneumoniae* and identified and confirmed by the use of microscopical, morohological, biochemical tests and Vitek 2 compact system. In the study of Adnan *et al.* (*2015*) in Yemen, in Sana'a city from June 2010 to January 2011, out of 150 samples from kidney transplantation patients, 50 were positive for GN bacteria. When Adnan's study is compared to our sample size and positive cases, the number is much higher and in the study of Mansury *et al.,* 2018) from Mashhad, Iran. Mansury et al., sampled 356 KTPs 127 positive for GN bacterial isolates. Also the number is much higher than our result even through there is no perceptible difference between the total numbers of samples.

In our study, *E. coli* 8 (4%) was the most common among others followed by *Klebsiella spp*. The E. coli number is lower than the result obtained by Mansury *et al*., (2018) where *E.coli* was 84 (66.1%) followed by *Klebsiella spp* 32 (25%) and it is lower than the result obtained by Gonen *et al*. (2018) in a study done in china in which *E.coli* 43 (42.2%) from a total of 70 isolated GN organisms. Also a higher number of *K. pneumoniae* was 9 (8.8%), isolates were higher than that of our result. Our result was lower than the result obtained by Adnan *et al.* (2015) in a study done in Yemen in which the most common isolated uropathogen was *E.coli* 22 (44%), and was 33% in the study of Shirazi *et al.* (2005*)* in Iran.

These differences in the numbers of *E coli* and *K pneumonia* may be due to the total size of the sample, the conditions in of the operation, the degree of aseptic techniques that are taken by the hospitals, the environmental conditions in which the patient stay after operation and/or the antibiotic profiles that are taken by the patients before and after the operation. These conditions are different from a hospital to another or from a city to another or between the countries making the differences in these numbers.

Among a total of 9 positive samples in our study, number of isolates in the urine sample 6 (66.66%) was higher than in the wound sample 3 (33.33%). There was no statistically significant correlation between 0.45 (P >0.05) GN bacteria and different clinical samples. Our results agree with the result reported by Leigh *et al*., (1969) in a study done in London where from (66) positive patients, the highest number of GN isolates was in urine 30 (45.5%) followed by wound 4 (6.1%). Also, in a study done in Mashhad Iran by Mansury *et al.* (2018), out of 164 positive cases, 128 samples were from urine followed by 10 samples from wound which confirms our results. These isolated organisms are often associated with certain and serious diseases in which UTIs are the most common problem after KT. Almost 70% of these infections are caused by GN bacteria, especially *E coli* and *K pneumoniae* (Ramadas *et al*., 2014), and are considered a potential cause of bacteremia, sepsis and affect graft outcomes (Yuan *et al*., 2018). Also wound infections are a major cause of post-operative morbidity in patients undergoing kidney transplantation (Harris *et al*., 2016). Also, sepsis is the main cause of mortality in non-cardiac invasive care units (Lagu *et al*., 2012). The number of isolates are higher in female samples 5 (55.56%) than in male samples 4 (44.44%) and statistical analysis shows no significant correlation between 0.34 (P >0.05) GN bacteria with gender. We compared these results with the result reported by Mansury *et al*. (2018) and the results were similar in with more isolates from female samples 67 (59.8%) compared with 45 (40%) in males (Adnan *et al*., 2015). These results contradict our result where males were 62% while female 38%. Also in a Medicare study of 28942 renal transplant recipients, the cumulative UTI in the first 6 post-transplant months was 17% in both genders. However, three-year post-transplant, the incidence in women increased to 60% while men were 47% with p< 0.001 (Abbott *et al.,* 2004).

Also, the female gender was associated with occurrence of UTI during hospital stay after the transplantation: 63% of females (n¼45/71) versus 35% of males (n¼42/121), P<0.001 (Maraha *et al*., 2001). Thus, female adults and children female are more vulnerable to UTI (Akbarzadeh *et al*., 2016).

The UTI variations between males and females in the GN isolates might be due to the sample size which was bigger in females than males (5, 4) respectively as our target populations consist of patients attending the hospital randomly.

Our results showed that the prevalence of *E coli* and *Kpneumoniae* in KTPs in the age of 10-19 years were (33.33%), followed by the age (80-89) years (22.22%). In the age of 30-39 and 40-49, and 50-59 years *E coli* and *Kpneumoniae* were 11.11%. Finally, from 20-29 and 60-69 years, there are no positive results contradicting the study of Chuang *et al.* (2005) where 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. There was no statistically significant correlation 0.11 (P >0.05) between GN bacteria with age. Also, the UTI risks in children and adult may differ. Overall, the risk of UTI in KT patients was 6-8 % based on the various UTI definitions and duration of follow up (Saemann *et al*.,2008). In the pediatric group, the UTI risk was 15-58% as John *et al* (2006) stated while in adult it was 61% (Veroux *et al*.,2008). According to Van der *et al*. (2006), UTI was high in children. It, in the first month following KT in children, was higher in terms of kidney damage (Dharnidharka *et al*., 2007). The analysis of urine culture in 109 (24 %) adults and 8 (13.8%) was positive (P>0.05) (Table -1). In this analysis, the most widespread organisms were *E. coli* in 63 (52%) in both genders which cause the positive urine culture. Also, *Entrobacter* was in 31(26%) patients. However, *E.coli* was only found in female children as Table -2 explains (P<0.05) (Akbarzadeh *et al*., 2016).

All *Escherichia coli* and *K. pneumoniae* isolates showed (88.89%) sensitivity to Meropenem, Amikacin, Imepenem and Ertapenem. followed by Tazobactum and Gentamicin, and statistical analysis showed a high significant

correlation 0.000003 ($p<0.05$) between GN bacteria with antibiotic susceptibility. In contrast, Amoxicillin and Ampicllin were the least effective antibiotics. However, this slightly contradicts a study because Ciprofloxacin was the most effective antibiotic while the current work showed that it was Meropenem with high resistance rates for *Escherichia coli* against Ciprofloxacin. In addition, there was a moderate susceptibility against Ciprofloxacin in this work for the same organism (Senger *et al*., 2007). Prophylactic antibiotics was administered to all the sample directly following surgery. In this study, the high resistance against antibiotic entailed that the infection may occur following the transplantation kidney for long periods and not by catheters. Elkehili *et al*. (2010) showed that Amikacin was highly resisted. Thus, the susceptibility to various isolates towards antibiotics in UTI was different according to countries and researchers deepening on therapy regimen and antibiotic misuse understanding on the one hand, and the researchers' use of standard antibiotic susceptibility testing technique on the other.

The two different hospitals in Erbil city used antibiotics Ampicillin and Amoxicillin and all bacterial isolated are 100% resistant 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. It could also be because they are very cheap, and so subject to abuse and misuse.

Our results showed that the majority of isolates of *E. coli and K. pneumoniae* were highly resistant to most antibiotics. On the other hand, *E. coli* and *K. pneumoniae* showed high resistance against Ampicillin, Ampicillin and Cloxacillin Amoxicillin. Also, out of 9 isolates 8 isolates showed resistance against Trimethoprime/ Sulphamethazaxole and Azithromycin. Moreover, some of the isolates were multiresistance to more than three antibiotics. Also, there was no statistical significant correlation 0.98 ($P>0.05$) between *E coli* and *Ka pneumoniae* and antibiotic resistances. On other hand, Klebsiella *pneumoniae* was totally 1 (11.1%) resistant to Azithromycin, Nitrofurontoin and Trimethoprim/Sulfamethazone, while the overall isolates of *Escherichia coli* and *Klebsiella pneumoniae* were multidrug resistant against more than three antibiotics. Out of 9 positive culture, most isolates were found to be MDR resistant to more than 4 antibiotics. Our finding are similar to the findings of Yuan *et al*'s (2018) in which out of 88 culture positive samples, 74 isolates (91.4%) were MDR.

Most MDR were prevalence in urine samples of *E. coli* and *Klebsiella pneumonia* in our results. The problem of resistance to Antimicrobial is increasing causing a great concern worldwide. Nearly antimicrobial agents are used to treat UTI in 1 of every 3 women who are younger than 24, while 40 % to 50 % of women will have UTI in their life. Over the previous ten years, the number of bacteria resisting antibiotic has increased universally. Like others, this study showed clear significant geographic variations in the vulnerability of the common antimicrobial agents to treat UTIs. It is important to understand antimicrobial resistance patterns among isolates of uropathogens for a clinically proper and cost-effective therapy (Aghazadeh *et al*., 2015).

There have been reported multi-drug resistant organisms such as ESB and/or Carbapenemase producing organisms in transplant units and could be linked to an appropriate prognosis (Linares *et al.*, 2007). The production of beta-lactamase enzymes is a very significant resistant mechanism in *E coli* and *K pneumonia* against betalactam antibiotics. Yazdi *et al*. (2012) stated that to treat bacterial infections, new brought-spectrum antibiotics like Cephalosporins have been used producing a new type of brought spectrum enzymes called beta-lactamase.

This study part of was conducted to analyze the effect of producing beta-lactamase from bacteria isolated from various sources. The result showed that ESBL was produced in 6 (66.66%) of every 9 *E coli* and *K pneumonia* isolates. *E. coli* (88.89%) was the highest incidence while *K .pneumoniae* was 0%. The statistical analysis showed no significant correlations between GN bacteria with ESBL. Also, our result were compared with other results and found that higher than study recorded by Vidal in the Spanish registry study; 26% of the 118 cases of symptomatic *E.coli* with UTI were caused by ESBL producing organisms (Vidal *et al*.,2012) Our data supports the evidence that *E coli* yielded the highest percentage of ESBL producers and all isolated of *E.coli* from most urine samples were generally resistant to the Ampicillin and Amoxicillin. *E. coli* now becomes the more common in multidrug resistant isolate and a problem of beta lactamases.

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