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Prevalence of Carbapenemase Genes in *Klebsiella pneumoniae* Isolates from Patients with Urinary Tract Infections in Baghdad Hospitals

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Abstract: The increasing incidence of carbapenems-resistant *K. pneumoniae* has been considered a public health problem especially among patients with urinary tract infections (UTIs) . Objective: The aim of this study was to evaluate the frequency of carbapenemase genes in *K. pneumoniae* isolates from UTIs and their role in the antibiotics resistance rate of these isolates. Material and methods: Eighty *K. pneumoniae* isolates were obtained from 250 urine samples (November 2020 to march 2021) from Baghdad hospitals, Iraq. Antimicrobial susceptibility was assessed by disc diffusion method. The carbapenemase genes *blaOXA48*, *blaVIM*, *blaIMP*, *blaKPC* and *blaNDM* were investigated by polymerase chain reaction (PCR). Results: *K. pneumoniae* isolates were detected in 32% of the tested samples in the patients with UTIs, and the prevalence of the isolates were higher in females in comparison with males. The results showed high resistance rates to Amikacin and Rifambicin (97.5%), to Cefotaxime, Ceftazidime and Piperacillin (92.5%), followed by Ceftriaxone and Aztreonam (87.5%), Cefepime (85%), Imipenem (62.5%), Meropenem and Gentamicin (50%). The most prevalent genes were *blaIMP* (37.5%), *blaOXA48* (30%), and *blaVIM* (28.75%), followed by *blaKPC* (20%), and *blaNDM* (11.25%). The most isolates which harbor the carbapenemase genes revealed a high resistance to cephalosporins and carbapenems. Conclusion: The rate of multi-drug and carbapenems resistance was high in the *K. pneumoniae* isolates from UTIs in Baghdad hospitals. Colistin could be the drug of choice for the treatments of carbapenemase-producing *K. pneumoniae*. Our study provides a better understanding of the carbapenemase genes distribution among the local isolates in UTIs.

Key words: *K. pneumoniae*, carbapenems-resistance , carbpenemase genes, UTIs.

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Introduction

Urinary tract infections (UTIs) represent one of the most widespread bacterial infections that require antimicrobial treatment (1). *Klebsiella pneumoniae* is an opportunistic pathogen and one of the most important bacteria in hospital acquired infections, causing soft tissue infections, septicemia, pneumonia, and urinary tract infections in patients admitted to hospital wards (2). The severity of Extended Spectrum Beta-Lactamase (ESBL) induced resistance in *K. pneumoniae* isolates has been described as a major public health threat. In addition to this, co-resistance among uropathogens to β-lactams, aminoglycosides, and fluoroquinolones further limits the available treatment options (3). The increased use of carbapenems has led to the emergence of

carbapenem resistance (CR) in Gram-negative bacteria (GNB), these CR pathogens are capable of spreading in the hospital setting and in the community (4).The rapid spread of CR has posed a global public health crisis owing to the lack of novel antimicrobials that could be used as an alternative last resort treatment (5). Carbapenem resistance in *K. pneumoniae* isolates is mediated by various mechanisms such as increased production of efflux pumps, reduced outer membrane permeability, changing tendency of the proteins bound to carbapenems, decreased production of porins, etc. However, in *K. pneumoniae* isolates, the major mechanism of acquired beta-lactam resistance is the production of beta-lactamases (6,7).

Carbapenemases are commonly found on mobile genetic components and have the potential to spread across the world (8). Most carbapenemase-producers (CPs) are MDR pathogens carrying multiple resistance determinants to other antimicrobial agents (9). The most common carbapenemases, based on carbapenem hydrolysis and geographical dissemination of outbreaks, are *K. pneumoniae* carbapenemases (KPC), New Delhi metallo-β-lactamase (NDM), imipenem-resistant carbapenemases (IMP), Verona integron-encoded metallo-β-lactamase (VIM), and oxacillinase (OXA-48-like) types. They are encoded by *blaKPC*, *blaNDM*, *blaIMP*, *blaVIM*, and *blaOXA-48* genes, respectively (10).

Therefore, the aim of this study was to evaluate the antibiotic resistance rate among *K. pneumoniae* local isolates recovered from patients with urinary tract infections at hospitals in Baghdad, Iraq, Also detection of the prevalence of carbapenemase genes .

Materials and Methods

The collection of study samples has taken place at the period between November 2020 and completed at end of march 2021, it included 250 clinical specimens as urine samples.

Isolation and identification of *K. pneumoniae*

This study was performed at Hospitals in Baghdad, Iraq, between November 2020 to march 2021. A total of 250 urine samples were collected from patients with UTI. McConkey agar and CHROM agar were used for isolating *K. pneumoniae*. These isolates were identified using traditional bacteriological methods and VITEK 2 system (bioMerieux, France), according to the manufacturer's recommendations.

Antibiotic Susceptibility Test

Antimicrobial susceptibility test was conducted by using disc diffusion method. Briefly, *K. pneumoniae* overnight growth were prepared on McConkey agar and then resuspended in Mueller-Hinton broth (Oxoid).The turbidity of the suspension was adjusted to an equivalent 0.5 McFarland and this suspension was used to inoculate on Mueller-Hinton agar (Oxoid) plates. The antibiotic discs used in this study as the following: Amikacin; AM, Rifambicin; RA, Cefotaxime; CTX , Piperacillin ;PRL, Ceftriaxone;CRO, Ceftazidime; CAZ, Azetreonam; ATM, Cefepime FEP, Ampicillin /sulbactam; AMP,

Imipenem;IMI, Ciprofloxacin;CIP, Meropenem ;MER, Gentamicin; GN, Trimethoprim TE, Tigacyclin TGC, Levofloxacin ;LEV, Tobramycin ;TOB, and Colistin; CL

The agar plates were incubated at 35 °C for 24 h. and then the inhibition zone was measured and isolate was interpreted by the percent susceptible, intermediate, or resistant as defined by CLSI breakpoint interpretative Criteria (11).

DNA Extraction and Identification of Carbapenem Genes by PCR

Bacterial DNA was extracted from all *K. pneumoniae* carbapenem producer isolates using ready kit (Promega, USA). Purity of the isolated DNA was monitored by NanoDropper 2000 (Thermo Scientific, USA). The PCR reactions for detection *blaOXA48*, *blaVIM* ,*blaIMP*, *blaKPC*, and *blaNDM* genes were done within a total volume of 20 µL. The mixture of reaction contained Master Mix (10 µL),1 µL of forward and reverse primers, DNA template 3 µL and Nuclease Free Water 5 µL . The Primer sequences, used for the detection of carbapenem genes in this study, were as in Table 1.

Table 1: Primer sequences for PCR detection of *K. pneumoniae* carbapenemase genes

Target Gene	Priymer name	Oligonucleotide primer Sequence (5′→3′)	Annealing Temp. (°C)	Amblicon size (bp)	References
blaKPC	KPC-F	CGTCTAGTTCTGCTGTCTTG	56	798	(12)
	KPC-R	CTTGTCATCCTTGTTAGGCG			
blaIMP	IMP-F	TTGACACTCCATTTACDG	55	139	(13)
	IMP-R	GATYGAGAATTAAGCCACYCT			
blaOXA48	OXA48-F	GCGTGGTTAAGGATGAACAC	52	438	(14)
	OXA48-R	CATCAAGTTCAACCCAACCG			
blaNDM	NDM-F	ATGGAATTGCCCAATATTATGCAC	52	813	(14)
	NDM-R	TCAGCGCAGCTTGTCGGC			
blaVIM	VIM-F	GAT GGT GTT TGG TCG CAT A	56	390	(13)
	VIM-R	CGAATGCGCAGCACCAG			

For the amplification of carbapenem genes, PCR conditions were carried out by the thermocycler (Applied Biosystems, Malaysia) according to the conditions of the previous studies as mentioned in Table (2). Agarose gel electrophoresis was done in a 1.2 % agarose gel at 80V for 2 hours. After electrophoresis fragments were stained by ethidium bromide or redsafe , and then visualized with ultraviolet light.

Table 2. PCR amplification conditions for carbapenemase genes in *K. Pneumonia* isolate

Steps	Temperature (°C)	Time/ sec	Cycles
Initial Denaturation	95	05:00	1 cycle
Denaturation	95	00:30	30 cycles
Annealing	52or 56 or 55*	00:30	
Extension	72	01:00	
Final extension	72	07:00	1 cycle

*Annealing of priemer 52 blaNDM and blaOXA48; annealing of priemer 56 blaKPC and blaVIM;annealing of priemer 55 blaIMP

Results and Discussion

collected from inpatients in four hospitals in Baghdad as the following (The Baghdad Teaching Hospital and Teaching Laboratories Institute-Medical city , Al-Yarmuk Teaching , Al-Kadhimiya Teaching Hospital and Al-Karama Teaching).

Isolation and Identification of *K. pneumoniae*

On MacConkey agar plates, *K . pneumoniae* isolates appeared mucoid, large and pink due to lactose fermentation, while on CHROM agar plates, colonies appeared as metallic blue colonies

Distribution of Samples among Patients

Only 80 (32%) samples showed significant growth of *K. pneumoniae* isolates, where the most of isolates were detected among females (n=58/ 72.5%), while the prevalence among males were 27.5% (n=22). The present study revealed that the highest percentage of *K. pneumoniae* isolates were from the individuals in the age group > 45 years followed by age group (35-45) years, and then (25-35) years, while the least age group of UTIs was (15-25) years (Figure 1).

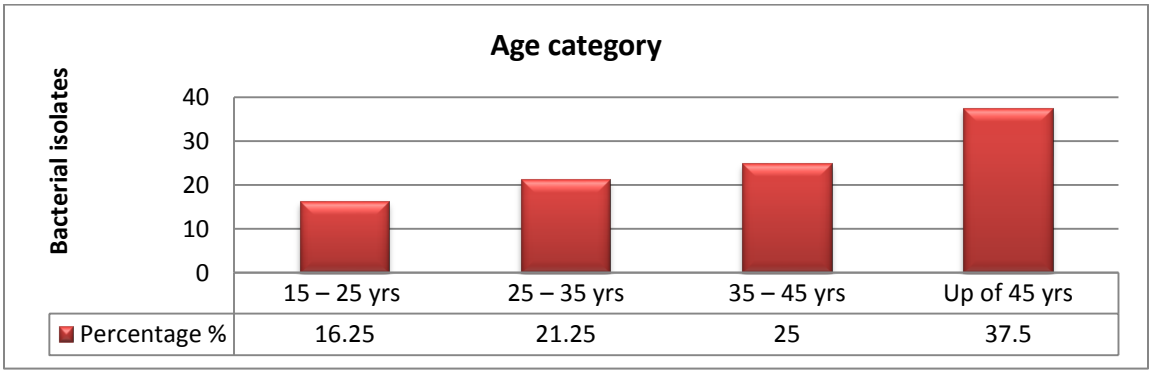

Figure 1: The distribution of *K. pneumoniae* isolates according to the age groups.

Although this infection affects both sexes, women are especially more prone to developing UTIs compared to the men this may be due to their anatomy and reproductive physiology that allows bacterial quick access to the bladder (15). One potential reason that could enable the auto-transmission and increasing the rate

of UTIs in women could be related to the closeness of the genital tract and the urethra and anus (16).

The highest percentage of *K. pneumoniae* isolates were from the individuals in the age range > 45 years (27.5%). This might be due to the UTIs prevalence that increases with catheterization, sexual intercourse and hormonal changes such as menopause (17). Indeed, *K. pneumoniae* comes after *E. coli* as the main cause of UTIs. Urinary tract infection was among females (64.3%) of males(46.5%) statistically higher in Zakho, Iraq(18) The current results demonstrated that the *K. pneumoniae* rate among the UTIs found to be 32%. Many studies revealed varied rates for *K. pneumoniae* such as 8.8% in Italy (15), 23.5% in Saudi Arabia (19). There are different pathogens associated with UTI, but the pathogenicity of *K. pneumoniae* was the highest which attributed to several virulence factors that give bacteria the ability to invade the host and prevent bacteria phagocytosis as well as forming a biofilm (20).

Our findings demonstrate that *K. pneumoniae* was prevalent among 72% of females and it was 27.5% among males. This result agrees with a study which revealed that female gender was more infected with uropathogenic *Klebsiella* than males (21). Also, it was reported by (22), in Pakistan, that men were more susceptible to uropathogenic *K. pneumoniae*. Additionally, (23), Korea, demonstrated that the rate of K. pneumoniae in UTIs was present in 7.7% of males and 5.3 in females.

This study revealed that patients within the age groupe (40-49) years have the highest prevalence rate with *K. pneumoniae*. This result agrees with a study by (24) and (25) Yang and Zhang who revealed that most *K. pneumoniae* were abundant among people in the age group of 18-65 and 40-65 years old respectively. (25) found that the age group above 70 years was with a high rate of this pathogen. On the other hand, Maglino et al.(15) revealed no significant differences in isolation rates within the age groups. The age-related differences in people could be due to the response of the immune system. Adult patients (under 40 years) are likely to have stronger immune systems. Also, elderly people with an increased incidence of illness will be at higher risk of *K. pneumoniae* infection (26).

Antibiotics susceptibility rate of *K. pneumoniae* isolates

The results of antibiotics resistance rate of all *K. pneumoniae* local isolates from UTIs against 19 antibiotics were summarized in figure 3.

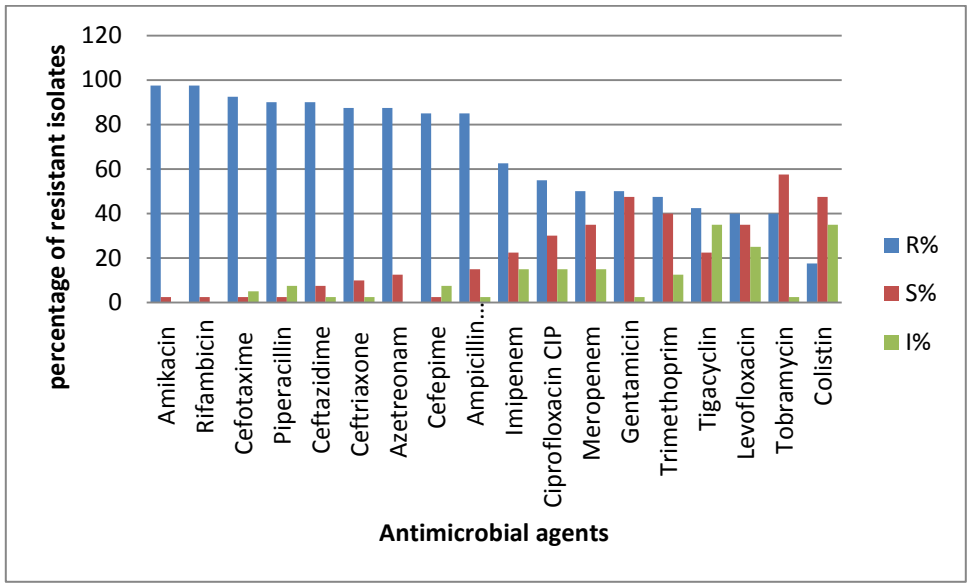

Figure 2: Antibiotics Susceptibility rate of the *K. pneumoniae* isolates from UTIs against different antibiotics. (Amikacin AM, Rifambicin RA, Cefotaxime CTX , Piperacillin PRL, Ceftriaxone CRO, Ceftazidime CAZ, Azetreonam ATM, Cefepime FEP, Ampicillin /sulbactam AMP, Imipenem IMI, Ciprofloxacin CIP, Meropenem MER, Gentamicin GN, Trimethoprim TE, Tigacyclin TGC, Levofloxacin LEV, Tobramycin TOB, and Colistin CL).

Results of the antimicrobial susceptibility test showed a high level of resistance towards the most of tested antibiotics. These findings indicated that most isolates were multi-drug resistant, where, the rate of resistance was (97.5%) to Amikacin and Rifambicin , (92.5%) to Cefotaxime, Ceftazidime and Piperacillin followed by (90%) to Ceftriaxone and Aztreonam (87.5%) ,Trimethoprim /sulfamethoxazole and Cefepime (85%), Imipenem (62.5%),Ciprofloxacin (55%), Meropenem and Gentamicin (50%),Trimethoprim (47%), Tigacyclin (42.5%), Levofloxacin and Tobramycin (40%)and Colistin (17.5).

The current study demonstrated the high resistance of *K. pneumoniae* isolates against aminoglycoides and cephalosporins. Several local studies reported a high rate of resistance of this bacteria to third and fourth generation of cephalosporines (27;28;29). This is consistent with the findings of many previous studies (30; 31). A study of (32) in the Kurdistan Region of Iraq revealed that all *K. pneumoniae* isolates (100 %) were resistant to cefepime, cefotaxime, and ceftazidime.

Carbapenems are the drugs of choice for many infections caused by *E. coli* and *K. pneumoniae* (33). In the present study, colistin and tigecyclinare the most effective antibiotics and the results indicated that most of the isolates were susceptible to these antibiotics, this is consistent with the findings of many previous studies. The emergence of carbapenems resistance in *K. pneumonia* is correlated with the production of *K. Pneumoniae* carbapenemase (KPC) (34). The increasing rates of drug-resistant isolates in Iraq may be due to the limited infection surveillance programs, limited laboratory facilities, the lack of

communication between physicians and microbiologists, lack of standardized criteria to determine drug-resistant isolates, and poor sanitation. The emergence of resistant *K. pneumoniae* bacteria is considered as an evidence of development of resistance, due to the possess mechanisms of resistance to carbapenems include production of lactamases and mutations that alter the expression and/or function of porins and PBPs (35).

The present results was in agreement with a study conducted In Iran which indicated that the antibiotic resistance patterns in clinical isolates of *Klebsiella pneumoniae* revealed widespread resistance to third-generation cephalosporins, with about half of them also resistant to gentamicin (37), also (38) mentioned that the antimicrobial drug susceptibility test of ESBL-producing *K. pneumoniae* isolates revealed high resistance rates for cefotaxime and ceftazidime (100 % and 88 %), as well as high sensitivity rates for colistin and tigecycline (100 % and 92 %). Several local studies revealed that the local *K. pneumoniae* isolates had a low-level resistance against Meropenem and Imipenem (28; 38). The findings of Wang et al.(39), indicated to the high resistance rate of carbapenems, 92.3% to imipenem and 93.2% to meropenem. Carbapenemases are commonly found on mobile genetic components and have the potential to spread across the world, (8). Most carbapenemase-producers (CPs) are MDR pathogens carrying multiple resistance determinants to other antimicrobial agents (9).

Detection of carbapenemases genes by Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from all *K. pneumonia* isolates. Extraction genomic DNA from 80 isolates that was confirmed as bands by gel electrophoresis. DNA concentration and purity were measured by Nanodrop spectrophotometer, all the isolates had DNA concentration between (50-100 ng/µl) and purity of the DNA were (1.4-2).

In order to detect the presence of carbapenemase genes (*blaKPC, blaVIM*, *blaIMP*, *blaNDM*, and *blaOXA 48*) and the determination of the prevalence of each gene among *K. pneumonia* clinical isolates, (PCR) for each DNA extracted sample have been used. The PCR products have been confirmed by the analysis of the bands on gel electrophoresis and by comparing their molecular weight with 100 bp DNA Ladder.

Each DNA extracted sample was subjected to PCR reaction with primer sets of *blaNDM* (813 bp), blaOXA48 (438bp), *blaKPC* (798bp), *blaVIM* (390bp) and *blaIMP* (139bp). The results of detection of these genes by PCR in all isolates were shown in figures 4 to 8, and the distribution of carbapenemase genes among 80 *K. pneumoniae* isolates from UTIs were demonstrated in the table 3.

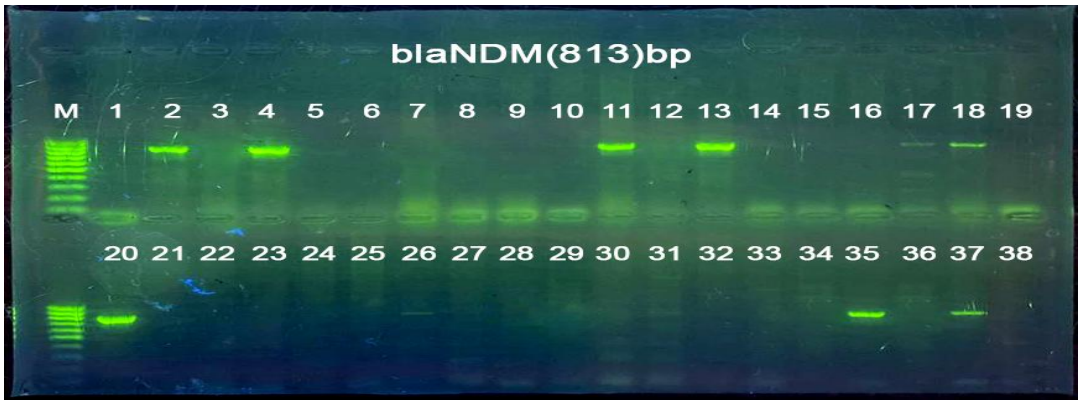

Figure 3: Agarose gel electrophoresis of PCR products for the resistance gene *blaNDM* (813bp). Lane M: 100bp DNA ladder; lanes 2-40: *K. pneumoniae* isolates; lane 1: negative control. (70V for 2hrs).

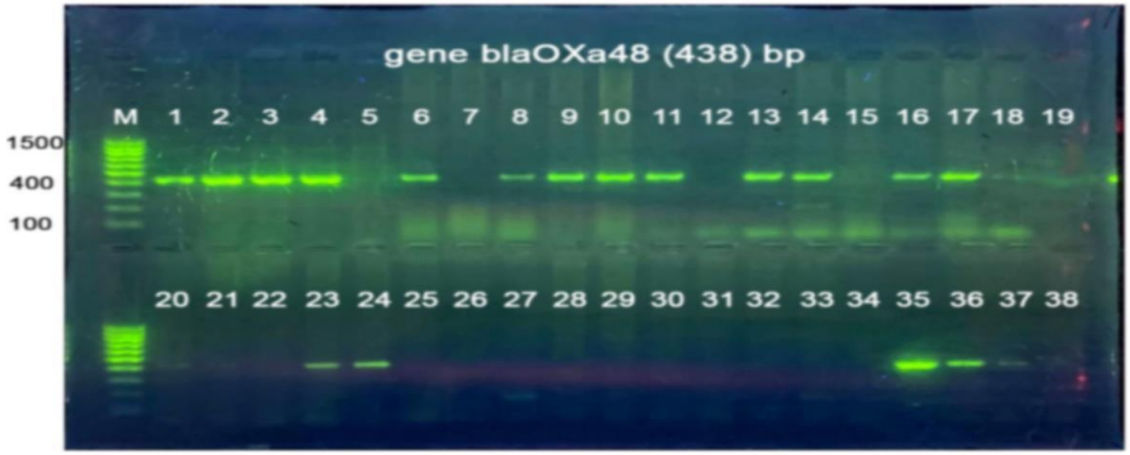

Figure 4: Agarose gel electrophoresis of PCR products for the resistance gene *blaOXA-48* (438bp). Lane M: 100bp DNA ladder; lanes 1-37: *K. pneumoniae* isolates ; lane 38: negative control. (70V for 2hrs).

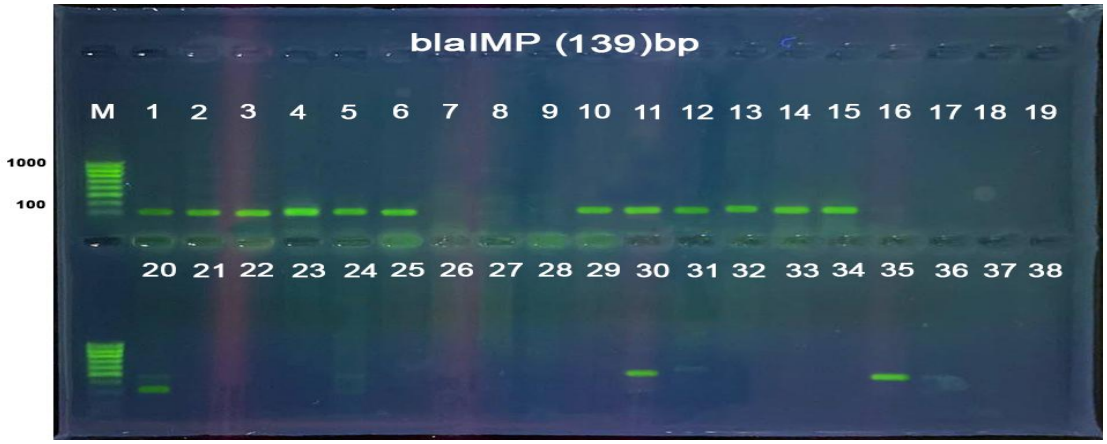

Figure 5: Agarose gel electrophoresis of PCR products for the resistance gene *blaIMP* (139bp). Lane M: 100bp DNA ladder; lanes 1-37: *K. pneumoniae* isolate; lane 38: Negative control. (70V for 2hrs).

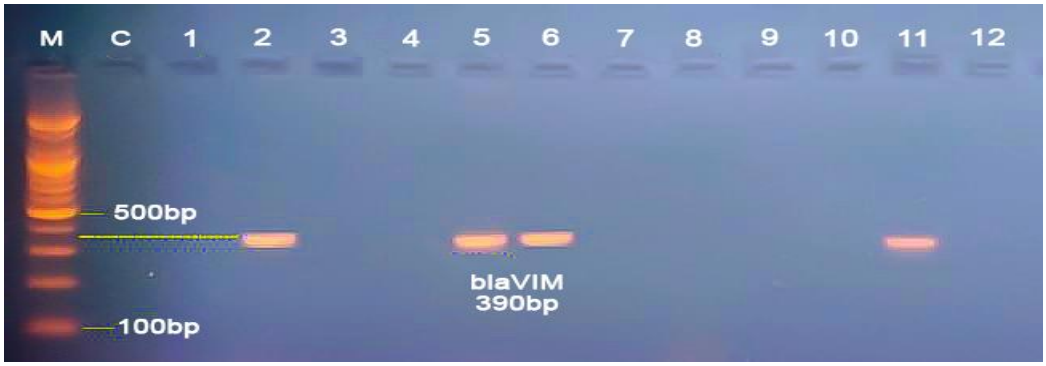

Figure 6: Agarose gel electrophoresis of PCR products for the resistance gene *blaVIM* (390bp). Lane M: 100bp DNA ladder; lanes 1-12: *K. pneumoniae* isolate; lane C: Negative control. (70V for 2hrs).

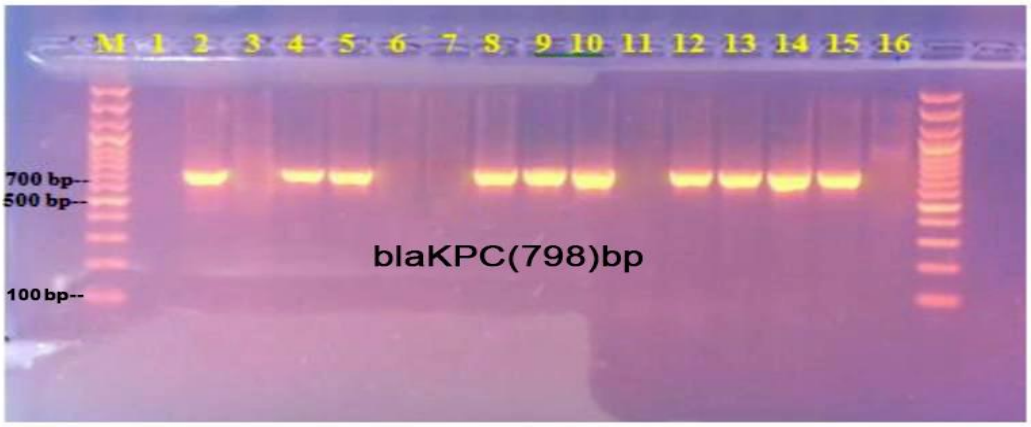

Figure 7: Agarose gel electrophoresis of PCR products for the resistance gene *blaKPC* (798bp). Lane M: 100bp DNA ladder; lanes 2-16: *K. pneumoniae* isolate; lane C: Negative control. (70V for 2hrs).

The results of PCR amplifications (Figures 4 to 8 and Table 3) revealed the detection and prevalence of carbapenemase genes among *K. pneumoniae* clinical studied isolates, these results exhibited that the main genotype in *K. pneumoniae* isolates from UTIs was *blaIMP* gene (37.5%) followed by *blaOXA48* genes (30%) while the lowest prevalence was for *blaNDM* gene (11.25%).

Table 3. The distribution of carbapenemase genes among 80 *K. pneumoniae* isolates from UTIs.

Gene	Number (%)
blaIMP	**30 (37.5%)**
blaOXA48	**24 (30%)**
blaVIM	**23 (28.75%)**
blaKPC	**16 (20%)**
blaNDM	**9 (11.25%)**

Beta-lactams, including carbapenems, are widely used in the treatment of Gram-negative bacterial infections, and it was obvious that the carbapenems, including imipenem and meropenem, have become the most effective drugs for the treatment of serious infections in hospitalized patients (40). However, resistance to these drugs has been reported by researchers in many cases. Carbapenem-resistant *K. pneumoniae*, due to the narrow therapeutic options and the high mortality rate, poses significant public health challenges worldwide (41). These results provide strong evidence that the *blaIMP*, *blaOXA48*, *blaVIM*, *blaKPC*, and *blaNDM* genes in *K. pneumonia*e are the genes responsible for resistance for carbapenem and production of carbapenemase. In the present study, the evaluation of drug resistance to carbapenems by the broth microdilution method showed that the resistance rates to imipenem and meropenem were 62% and 50%, respectively.

The *K. pneumoniae* carbapenemase (KPC) enzyme is the most concerning Ambler class A because of its location on self-conjugative plasmids and ability to transfer resistance among Enterobacteriaceae. This enzyme is able to hydrolyze penicillins, classical cephalosporins, monobactam, and all carbapenems such as imipenem, meropenem, ertapenem, and doripenem, and it is weakly inhibited by clavulanic acid and tazobactam (41). The enzymes of the VIM, IMP, and NDM groups are in Ambler class B, and their genes are located on the plasmid. These enzymes cannot be physically bound to the beta-lactam substrate and therefore escape the action of beta-lactamase inhibitors such as clavulanic acid and sulbactam and are able to bind to all beta-lactam classes, except monobactam (42). OXA-type carbapenemase enzymes are also in the D class of Ambler and are known as oxacillinases due to their accelerated hydrolysis of classical penicillins. The bla OXA-48 enzyme, is one of the most important and common enzymes in the development of carbapenem resistance, and its gene is on the plasmid (43).

The present findings did not agree with a local study conducted on hospitalized patients in Baghdad(44), The phenotypic detection of carbapenemases revealed that 55 were carbapenem-resistant *K. pneumoniae* strains. The result showed 37 (67.27%) strains positive for blaNDM-1 gene and only 5 (9.1%) strains harbored blaIMP gene (45). The study of (46), who showed that the production of OXA-48 is one of the main mechanisms of resistance to carbapenems in carbapenem-resistant *K. pneumoniae* (CRKP) isolates in Isfahan. In addition, the dissemination of NDM-producing CRKP isolates is a potential risk for the health care systems. In Egyptian study the results of PCR showed that carbapenem resistance may be due to NDM gene that was present in 43% of the isolates and OXA gene that was found in 28% of the isolates and the results showed increased resistance to various antimicrobial agents and also high MIC values for imipenem and meropenem, where this may be markedly attributed to various resistance mechanisms that led to resistance to most antimicrobial classes (47).

In Pakistan, the study among CRKP, revealed that 91 (77.8%) of isolates were detected as carbapenemase producers, while 55(47%) were positive for *blaNDM-1* and 22.2% (n=26) of the isolates were positive for *blaOXA-48* (48).

In conclusion, carbapenem-resistant *K. pneumoniae* contribute in urinary tract infections, and their antibiotics resistance may be due to expressed multiple drug resistance genes, especially carbapenems resistance genes. In the present study, *blaIMP* and *blaOXA48* were the predominating resistance genes. carbapenem-resistant *Klebsiella pneumoniae* is a severe threat to the healthcare systems in our local hospitals.

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