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Detection of *blaOXA-48* and *blaVIM-1* Genes among Carbopenems Resistance in *Klebseilla Pneumoniae* Isolated from Urinary Tract Infections in Baghdad Hospitals

Zeaid H. Aboud[1], Abdul Muhsin M. Shami[*2], Bushra A. Abdul Ridha[3]

[1,2]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

[3]Diagnostic on AMR unit, CPHL in Baghdad

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Abstract: *Klebsiella Pneumoniae* gram-negative bacteria are common causes of urinary tract infections (UTIs). Such pathogens can acquire genes encoding multiple mechanisms of antimicrobial resistance, including carbapenem resistance. This study aimed to detect the carbapenemase-producing ability of some *K.pneumoniae* bacterial isolates from urine specimens of patients suffering from complicated UTIs at three vital care hospitals in Baghdad, Iraq; and determine the prevalence of carbapenemase genes among plasmid-bearing isolates, and explore the possibility of horizontal gene transfer to other bacterial species. The samples were collected from November 2021 to April 2022. About180 samples of urine were collected from in patient's and outpatients who attended they were collected at three Baghdad hospitals: two from Karkh (AL-Yarmouk hospital and Al-karamu hospital), and one from Rusafa (Baghdad educational hospital). Most of the patients were females (62.7%), while the percentage of males was (37.2 %). Midstream urine MSU samples were collected in sterile containers from patients with symptoms of urinary tract infection. The samples were cultured on blood agar and MacConkey agar, Chroma ager selective media and incubated at 37℃ for 24 hr. The results were recorded by observing grown colonies and isolating Gram-negative bacteria. In this study, all n=21 (100%) isolates were determined positive for the *16srRNA* housekeeping gene, n=11(52.38%) isolates were positive for the *blaOXA-48* gene, and n=6 (28.57%) isolates were positive for *blaVIM-1*, The extracted plasmids were used as templates for PCR amplification of *blaVIM-1*, and *blaOXA-48* carbapenemase genes. Plasmids carrying the *blaOXA-48*, and *blaVIM-1*genes from six *K. pneumoniae* clinical isolates were successfully transformed into competent. The transformants were carbapenemase-producers and acquired resistance to some of the tested antimicrobial agents as compared to untransformed ones. The study concluded that the rate of carbapenem resistance among *K.pneumoniae* bacterial uropathogens in Baghdad, is relatively high and can be transferred horizontally to other bacterial hosts.

Keywords: *blaOXA-48; blaVIM-1*; *Klebsiella pneumoniae*; carbapenem-resistant.

Corresponding author: (Email: zeyed.Hafez1200a@ige.uobaghdad.edu.iq).

Introduction

Klebsiella pneumoniae is a facultative anaerobic bacterium that is Gram-negative, encapsulated, rod-shaped, non-motile, lactose-fermenting, gas-producing, and prolong to the *Enterobacteriaceae* family of Gammaproteobacteria. *K. pneumoniae* may develop in the presence or absence of oxygen, making it a facultative anaerobe (1). It causes several diseases including pneumonia, urinary tract infections (UTIs), bloodstream infections, and sepsis. Which infections

is particularly a problem among neonates, elderly and immunocompromised individuals, *Klebsiella* is also responsible for a significant number of community-acquired infections (2).

Urinary tract infection is one of the most common infectious diseases in developing countries (UTI). Antibiotic resistance has emerged as a result of the widespread use of antibiotics to treat uropathogens (3). Because of its various impacts on the urinary tract and host immune system, urinary tract infection (UTI) is a serious health concern in diabetes people. Patients with an atypical genitourinary tract are more likely to have complicated UTIs. To avoid morbidity and life-threatening conditions linked with UTI, a thorough evaluation and rapid treatment are required (4).

Antibiotic resistance among Extended-Spectrum Beta-Lactamases (ESBLs)-producing uropathogenic bacteria to the most popular cephalosporins used in Iraqi hospitals has been reported in several local investigations (5). The genetic identification of ESBL-producing organisms is vital for epidemiological application because the numerous Extended-Spectrum Beta-Lactamases (ESBLs) expressing genes in bacteria may disclose typical characteristics with antimicrobial resistance expression (6). Treatment of multidrug-resistant bacteria has proven to be a therapeutic challenge, and Extended-Spectrum Beta-Lactamases (ESBL)-producing isolates are regarded as serious public health, and financial challenge because physicians are limited in their choice of appropriate antibiotics for effective treatment of ESBL infections, they're objectives are to currently study, we aimed to investigate the presence of *blaOXA-48* and *blaVIM-1* genes (7).

Urinary tract infections, which account for around 3% of all infections in children, are a significant healthcare issue. Rising antibiotic resistance is a subject of increasing reports, which also highlight the need for ongoing monitoring of antimicrobial efficiency, particularly in places where widespread drug abuse is prevalent. Analyzing bacterial isolate resistance to commonly taking medication in pediatric populations is the goal of this retrospective study (8).

The development of carbapenem-hydrolyzing-lactamase is primarily responsible for *K. pneumonia* resistance to carbapenems. Recent research has been done on the prevalence of *KPC, OXA-48,* and Verona Integron-encoded Metallo-lactamase (*VIM-1*), generating strains of *K. pneumoniae* in various nations. Because these genes are found on mobile genetic components like transposons and plasmids, transmission to other gram-negative bacteria should be taken into account (9).

Materials and methods

Sample collection

The samples were collected from November 2021 to April 2022. About180 samples of urine were collected from inpatient and outpatients who attended they were collected at three Baghdad hospitals: two from Karkh (AL-Yarmouk Hospital and Al-Karamu Hospital), and one from Rusafa (Baghdad Educational Hospital). Most of the patients were females (62.7%), while the percentage of males was (37.2%).

Midstream urine MSU samples were collected in sterile containers from patients with symptoms of urinary tract infection. The samples were cultured on

blood agar and MacConkey agar, Chroma ager selective media and incubated at 37℃ for 24 hr. The results were recorded by observing grown colonies and isolating Gram-negative bacteria.

Culture

All urine samples (midstream urine and after cleaning the genitals) were collected in sterile disposable containers (HI media-India) and centrifuged (Memmert-Germany) at 2000 rpm for 2 min. Immediately, the sediment was incubated with brain heart infusion broth (Oxoid-UK) at 37^{o}C overnight and streaked by sterile swab (Bioanalyses-Turkey) on blood agar (Oxoid-UK) surface and MacConkey agar (Oxoid-UK) surface and incubated aerobically overnight at 37C^{o}. In the laboratory of each hospital under aseptic conditions, the collected specimens were streaked directly on Chrome agar medium, MacConkey agar and blood agar then incubated for 24 hours at 37°C, under aerobic conditions. The metallic blue colonies of *K. pneumoniae* on chrome agar medium, Pink, and lactose fermenting colonies on MacConkey agar were sub-cultured on another MacConkey agar plate and incubated for another 24 hours at 37°C and showed non-hemolytic grey-white, mucoid colonies when plated on blood agar. Further identification tests included morphological characteristics and biochemical tests were carried out.

Antibiotic susceptibility test

The antibiotic susceptibility test was carried out using the Kirby-Bauer method, as described by (10). Meropenem and imipenem antibiotics were subjected to an antibiotic susceptibility test. Picking 1-2 isolated colonies of *K. pneumoniae* from the original culture and introducing them into a test tube containing 4 ml of normal saline resulted in a moderate turbidity bacterial suspension. It was compared to a conventional turbidity solution (0.5 McFarland), which has about $1.5x10^{8}$ CFU/ml. A quantity of the bacterial solution was gently and evenly placed over Mueller-Hinton agar medium using a sterile cotton swab and then left for less than 5 minutes. Following that, the antimicrobial discs were placed on the agar with sterile forceps pressed firmly on the agar to ensure contact. Later the plates were inverted and incubated at 37ºC for 18-24 hours. According to the Clinical and Laboratory Standards Institute, inhibition zones produced around the discs were measured in millimeters (mm) using a metric ruler (10). By comparing the isolates to typical inhibitory zones, the isolates were classified as susceptible, intermediate, or resistant to the antibiotic. In the present study, a total of (n=50) *K. pneumoniae* clinical and hospital environment samples collected from several hospitals resulted in the emergence of multiple drug-resistant, which became a major challenge in the treatment of corresponding infections clinically. Therefore, antibiotic susceptibility of the bacterial isolates was performed on antibiotics represented by The Thirteen antimicrobials listed below were utilized.

Ceftazidime (CAZ), Cefotaxime (CTX), Ceftriaxone (CRO), Trimethoprim (TMP), Amoxicillin / Clavulanate (AMC), Nitrofurantoin (F), Nalidixic acid (NA), Gentamicin (GM), Imipenem (IPM), Meropenem (MEM), Tobramycin (TOB), Ciprofloxacin (CIP), Tetracycline(TE) using the disc diffusion method.

Primers design, selection, and preparation

Concerning the conventional PCR reaction, selected primers were used for detecting *K. pneumoniae* for each gene. The forward and reverse primers detecting sizes (389, 743bp) fragments of the *blaVIM-1like* gene, *blaOXA-48* gene, and size (150bp) fragment of *16srRNA* genes, respectively, were according to the methods described by (11,12,14). Table (1) listed the sequences of the studied primers.

Identification of *K. Pneumoniae* and carbapenemase resistance genes by molecular method

DNA extraction

The method (13) was used for the extraction of total DNA as follows: Five pure and fresh colonies of *K. pneumoniae* strains were suspended in (200 µl) of sterile deionized water, and cells were placed in the water bath (Memmert-Germany) at 100C^{o} for 30 min, immediately the solution was placed in ice for 30 min and the other cellular components were removed by centrifugation at 9000 rpm for 15 min. Finally, the supernatant was used as the DNA template.

Table (1): Sequences of the primers used for conventional PCR to detect *K. pneumoniae blaOXA-48, blaVIM, and 16srRNA* β-lactamase genes

Gene	Oligonucleotide primer sequence 5' to 3'	PCR product size (bp)	Reference
***bla*$_{VIM-1}$**	F: GTT TGG TCG CAT ATC GCA AC	389	(11)
	R: AAT GCG CAG CAC CAG GAT AG		
***bla*$_{OXA-48}$**	F: TTG GTG GCA TCG ATT ATC GG	743	(14)
	R :TGAGCTTCTTTTGTGATGGCT		
16s rRNA	F: CAGCTCGTGTCGTGAGATGT	150	(12)
	R: CGTAAGGGCCATGATGACTT		

*** F: Forward sequences, *R: Reverse sequences.**

(Alpha DNA-Canada) primers were commonly shipped in a lyophilized state. The units of a lyophilized primer were given as a mass, in Pico moles. To create a stock of primers, The following steps were followed for reconstituting and diluting the primers:

1- Spin down the tube before opening the cap.
2- The desired amount of water was added according to the oligo's manufacturer to obtain (100 p moles /µl) (Master Stock).
3- Vortex properly to re-suspend the primers evenly.
4- Transferred 10 µl of the master stock to a 0.2 µl Eppendorf tube that contains 90 µl of sterile, nuclease-free H2O (Working Stock).
5- The master stock was stored at -20 ^{o}C.
6- The working stock was stored at -20 ^{o}C.
7- The working stock was thawed on ice and vortex before using in PCR and then stored at -20 ^{o}C.

PCR

Using 25µL of PCR reaction, 1.5 µl DNA template (60 ng/µl) was amplified using 12.5 µl of G_2 Go *Taq*® green master mix (Promega/ USA) and 1.0 µl of each primer (10 pmol/µl) of the gene, up to the final volume 25 µl with nucleases free water Table(2). The

extracted DNA, primers, and PCR premix were thawed at 4°C, vortexed, and centrifuged briefly to bring the contents to the bottom of the tubes. Optimization of the polymerase chain reaction was accomplished after several trials. The negative control contained all material except DNA, so nuclease-free water was added instead of template DNA run. PCR programs were set on Thermal-cycler (Applied Biosystems / USA). PCR Thermocycler quantities: PCR Thermocycler quantities were performed by using the conventional PCR Thermocycler described in Tables (2) and (3).

Table (2): Component of PCR master mix reaction

PCR Master mix reaction components		Volume
Master Mix or GoTaq ® Green Master Mix		12.5 µl
DNA template		1.5 µl
Primers	Forward	1µL
	Reverse	1µL
nuclease-free H_2O		9µL
Total volume		25µL

Klebsiella. pneumoniae was identified thanks to the discovery of the *16s rRNA* gene. The protocol for PCR amplification was developed after multiple experiments, as shown in Table (3). PCR Thermocycler conditions were done by using conventional PCR Thermocycler as in tables (4), (5).

Table (3): PCR Thermo-Cycling program for *16S rRNA* gene

Loop's steps	Temperature	Time	Number of cycles
Initial denaturation	95ºC	5 min	1
Denaturation	95ºC	45 sec	30
Annealing	60 ºC	45 sec	
Extension	72 ºC	45 sec	
Final extension	72 ºC	5 min	1

Table (4): PCR Thermo-Cycling program for *blaOXA-48* gene

Loop's steps	Temperature	Time	Number of cycles
Initial denaturation	95ºC	5 min	1
Denaturation	95 ºC	45 sec	30
Annealing	50 ºC	45 sec	
Extension	72 ºC	45 sec	
Final extension	72 ºC	5 min	1

Table (5): PCR Thermo-Cycling program for *blaVIM-1* Gene

Loop's steps	Temperature	Time	Number of cycles
Initial denaturation	95ºC	5 min	1
Denaturation	95 ºC	45 sec	30
Annealing	59 ºC	40 sec	
Extension	72 ºC	45 sec	
Final extension	72 ºC	5 min	1

Statistical analysis

The statistical analysis System-SAS (17) program was used to detect the effect of different factors on study parameters. Least significant difference –the LSD test (Analysis of Variation-ANOVA) was used to compare significant means. The Chi-square test was used to compare significant percentages (0.05 and 0.01 probability) in this study (17).

Results and discussion

Description of the study samples the specimens of this study were collected from November 2021 to April 2022. The study included around 180 clinical samples taken from midstream urine (MSU). Patients with urinary tract infections, both outpatients and inpatients (UTIs). The administration samples must be in sterile containers. Most of the patients were females n=113 (62.7%), while the percentage of males was n=67 (37.2%). The specimen is classified according to age and gender as shown in table (6).

Table (6): Distribution of specimen collection from patients with UTI according to age and gender

Age	Male	Female	Total	%
1 to 5 years	20(11.1%)	18(10%)	38	21.1%
6 to 10 years	10(5.55%)	20(11.1%)	30	16.6%
11 to 20 years	12(6.66%)	26 (14.4%)	38	21.1%
21 to 30 years	9(5.0%)	21(11.6%)	30	16.6%
31 to 40 years	7(3.88%)	15(8.33%)	22	12.2%
41 to 50 years	3(1.66%)	7(3.88%)	10	5.5%
51 to 60 years	4(2.22%)	5(2.77%)	9	5.3%
61 to 80 years	2(1.11%)	1(0.55%)	3	1.6%
Total	N=67 (37.3%)	N=113 (62.7%)	180	100%

Isolation and identification of *K. pneumoniae*

According to Parven *et al.* (18), following morphology identification by Gram's staining, cultural properties, and biochemical characteristics, samples were isolated and identified. The growth and appearance of *K.pneumoniae* in culture media used for it are as follows:

Streaking all the samples (clinical and hospital environmental samples) on MacConkey agar, Blood agar, and CHROMagar, and incubated at 37°C overnight for 18 to 24 hours. The following characteristics of the isolates grown from these media were used to identify them.

The positive result for *K.pneumoniae* on MacConkey agar is typically displayed on formed bright pink colonies with a mucoid structure due to fermenters of the lactose sugar in the media, which is a feature of this bacteria. While blood agar appeared as non-hemolytic grey-white colonies, cultivated on blood agar, they did not produce blood hemolysis, which is a feature of this bacteria (19).

CHROMagar *Klebsiella* medium

CHROMagar medium was used for specific isolation of urinary tract pathogens. On CHROMagar, isolates of *Klebsiella* appeared as metallic blue colonies at 37°C for 24 hours as shown in Figures (1).

This medium also has selectivity for other Urinary tract pathogens with a

specific color of metallic blue colonies of *K.pneumoniae*, this medium also has selectivity for multidrug-resistant *K. pneumonia* by adding the supplement (CR102) to the prepared medium (20).

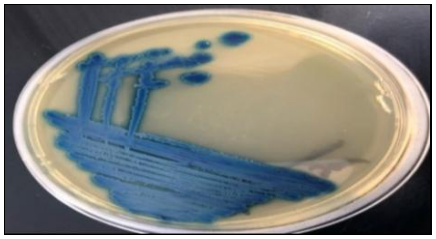


Figure (1): Colonies of *K.pneumoniae* on CHROMagar Orientation medium.

All of our samples were cultured on CHROMagar *Klebsiella*, a highly selective and sensitive media for *K.pneumoniae* that contains compounds that prevent gram-positive bacteria from producing gram-negative bacilli. It contains substrates that enable color-based preliminary colony identification between (18-24) hr. following inoculation. About 50 isolates from *K.pneumoniae* were confirmed on the CHROMagar, which is a diagnostic and differential media between the isolated,

Distribution of bacterial strain isolated from UTI

The positive results of the API 20E System and the VITEK 2 System on the samples of the current investigation all (180 samples) were n=50 samples isolated from various clinical in the infection of UTI. Depending on the UTI infection that has been taken.

The distribution of pathogenic bacteria isolated from urine samples that cause UTI infection in our investigation is shown in Table (7).

Table (7): Distribution and percentages of bacterial strain isolated collection from patients with cases of urinary tract infection

Microorganisms	No. of isolate	Percentage%
Klebsiella pneumoniae	50	27.78%
Escherichia coli	33	18.34%
Pseudomonas aerugenes	8	4.44 %
Enterobacter cloacae	6	3.33%
Proteus mirabilis	4	2.22 %
Proteus vulgaris	3	1.67%
Staphylococcus aureus	2	1.11%
Candida albicans	2	1.11%
No growth	72	40%

Table (7) shows that *K.pneumoniae*, the most prevalent isolate, was found to account for around a quarter of all samples isolated (n=50) (27.78 %). Meanwhile, *E. coli* UTI isolates made up a significant portion of the samples (n=33) (18.34 %). In addition to *K. pneumonia*, many other species were detected in the total samples examined. *E.coli* and *Klebsiella spp*. were the bacteria most commonly found in clinical samples. Second infections, are *Pseudomonas aeruginosa* n=8 (4.44%), followed by *Enterobacter cloacae* n=6 (3.33%), *Proteus mirabilis* n=4 (2.22%), *Proteus Vulgaris* n=3 (1.67%), *Staphylococcus aureus* n=2 (1.11%), and *Candida*

albicans n=2 (1.11%) was found in diverse amounts and percentages *K. pneumoniae*, which causes urinary tract infections. These results agree with(21), that is the second most common pathogen in hospitals. *K. pneumoniae* is one of the most common bacteria, according to several studies.

Antibiotic susceptibility of *Klebsiella pneumoniae*

Thirteen different antimicrobials were used in this study, according to the Kirby-Bauer method, and the Clinical Laboratory Standards Institute (CLSI) was used as a guideline zone diameter and MIC breakpoints *Enterobacterales* as follows: media. direct colony suspension From *K. pneumoniae* strain was cultured in Mueller Hinton agar and MacConkey agar. *K. pneumoniae* cultures were adjusted using the 0.5McFarland, fresh colonies (18-24hr), incubation at 37°C, ambient air, for (16-18 hr.) for disc diffusion, for (16-20hr) dilution methods, alerts in case of detection of CRE and ESBL isolates .streaked over the Mueller Hinton agar surface using a sterile brush. According to CLSI guidelines 2021. Strain growth zone diameter was used to determine antimicrobial sensitivity and resistance.

In the present study, a total of (n=50) *K.pneumoniae* clinical and hospital environment samples collected from several hospitals resulted in the emergence of multiple drug-resistant, which became a major challenge in the treatment of corresponding infections clinically. Therefore, antibiotic susceptibility of the bacterial isolates was performed on thirteens' antibiotics represented by The Thirteen antimicrobials listed below were utilized. Ceftazidime (CAZ), Cefotaxime (CTX), Ceftriaxone (CRO), Trimethoprim (TMP), Amoxicillin / Clavulanate (AMC), Nitrofurantoin (F), Nalidixic acid(NA), Gentamicin (GM), Imipenem (IPM), Meropenem (MEM), Tobramycin (TOB), Ciprofloxacin (CIP), Tetracycline(TE) using the disc diffusion method in the table (8).

Table (8): Antimicrobial susceptibility test of 50 *K. pneumonia* isolates

Antibiotic	No. isolated	R	No. isolated	I	No. isolated	S
MEM	21	42%	0	0%	29	58%
IPM	18	36%	3	6%	29	58%
AMC	48	96%	0	0%	2	4%
CAZ	35	70%	5	10%	10	20%
CIP	28	56%	2	4%	20	40%
CN	20	40%	0	0%	30	60%
CRO	35	70%	0	0%	15	30%
CTX	30	60%	10	20%	10	20%
F	23	46%	22	44%	5	10%
NA	15	30%	7	14%	28	56%
TE	50	100%	0	0%	0	0%
TMP	50	100%	0	0%	0	0%
TOR	15	30%	2	4%	33	66%

***R: Resistance, I: Intermedia, S: Sensitive.**

Table (8) shows for the isolates under study revealed that of key clinical isolates are resistant to tested antibiotics at high levels. It was found that all isolates of *K. pneumoniae* were resistant to Trimethoprim and Tetracycline (100%). As the present study showed the highest resistance to Amoxicillin/ Clavulanate (96%), Ceftazidime, and Ceftriaxone (70 %), also high resistance

was recorded for the antibiotics; Cefotaxime (60%), Ciprofloxacin (56%), Nitrofurantoin (46%), Meropenem (42)%, Gentamycin (40 %) and Imipenem (36%). Moderate resistance was observed for Nalidixic acid, and

Tobramycin (30%). The current study demonstrated that *K. pneumoniae* possessed a low-level resistance against it, statistically, there was a highly significant difference ($P \leq 0.01$).

K. pneumonia's resistance to numerous antibiotics, resulting from excessive antibiotic administration, is now leading to the accumulation of antibiotic resistance and cross-resistance between antibiotics and the appearance of multi-drug resistant MDR forms of *K. pneumoniae* (22).

In the present study, ten isolates of *K.pneumoniae* have been chosen which were multi-drug resistant as shown in figures (2).

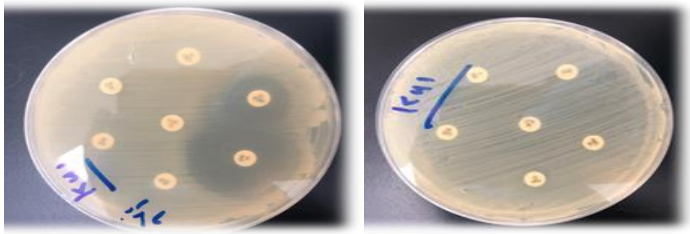
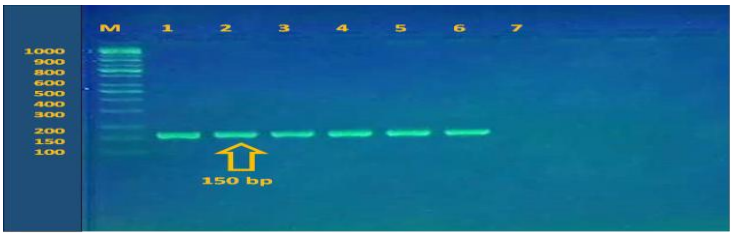


Figure (2): Antibacterial susceptibility of *K. pneumoniae* isolates against 13 antimicrobial agents

Molecular detection of *blaOXA-48, VIM-1,* and *16srRNA* in *K. pneumonia*

The study looked at a total of n=21 carbapenem-resistant isolates. These isolates were found in clinical samples. Urine samples account for the majority of the clinical specimens (180). Isolates (n=21) tested positive for meropenem and imipenem resistance. Furthermore, Fifty *K.pneumoniae* isolates and (n=21) isolates were found to be resistant to imipenem and or intermediate to meropenem, respectively. They used to determine the prevalence of Extended Spectrum Beta-Lactamase genes (*blaOXA-48, blaVIM-1*), and *16srRNA* housekeeping gene among *K. pneumoniae* clinical isolates, we used a protocol for the kit (Promega /USA) and amplification of the DNA sample by (Thermocycler/USA) polymerase chain reaction (PCR) for each of our isolated (n=21) DNA extracted samples. By examining the bands on gel electrophoresis and comparing their molecular weight to a 100 bp DNA ladder, the PCR products were confirmed.



Figure(3): Agarose gel electrophoresis of PCR products for *16s rRNA* gene size (150 bp). Lane (M): l00bp ladder, Lane (1-6): *K. pneumoniae*; Lane 7: Negative control (70V for 1hr).

As to determine the prevalence of each gene among *K. pneumoniae* clinical isolates, conventional polymerase chain reaction PCR (Thermocycler/USA) was used to detect the existence of *16srRNA* and

carbapenem-resistant genes (*blaOXA-48* and *blaVIM*-1) and gene size of (150bp), (743bp and 389bp), respectively for each DNA extracted sample. Checking the bands on gel electrophoresis and comparing their molecular weight to that of the 100 bp DNA ladder validated the PCR results. The findings of a standard PCR assay 16srRNA and for the *blaOXA-48* and *blaVIM-1* genes are shown in figures (3), (4) and (5).

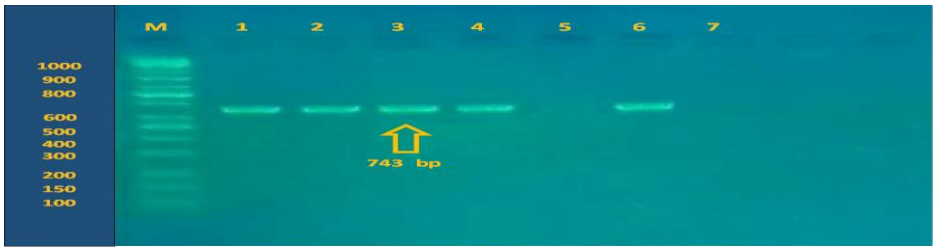


Figure (4: Agarose gel electrophoresis of PCR products for *OXA-48* Gene size (743 bp). Lane (M): l00bp ladder, Lane (1- 6): *K. pneumoniae* with; Lane 7: Negative control (70V for 1hr).

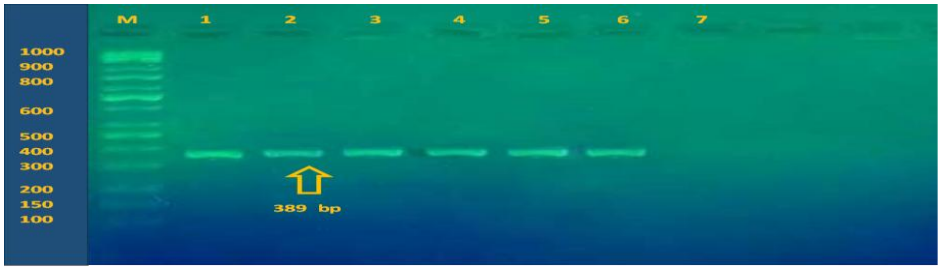


Figure (5): Agarose gel electrophoresis of PCR products for *blaVIM-1* gene size (389 bp). Lane (M): l00bp ladder, Lane (1- 6): *K. pneumoniae* with; Lane 7: Negative control (70V for 1hr).

In this study, all n=21 (100%) isolates were determined positive for the *16srRNA* housekeeping gene and 11(52.38%) isolates were positive for *the blaOXA-48* gene. And 6 (28.57%) isolates were positive for *blaVIM-1*. The optimized thermocycler PCR assays enhance the positive diagnostic rate compared to the conventional bacterial culture approach, and also quickly detected important associated genotypes that confer beta-lactam resistance for bacteria (23).

Conclusions

This study shows that *K. pneumoniae* resistance to carbapenems, one of the last resort classes of antibiotics, became prevalent and distributed among Gram-negative bacterial uropathogens in three major tertiary care hospitals in Baghdad, Iraq. This type of resistance can be transferred horizontally to other bacterial hosts causing limitations and challenges in treatment options for bacterial infections. Antibiotic stewardship programs must be implemented to reduce the emergence and spread of CR and improve the outcomes of infectious diseases treatment programs.

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