AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN: 2706-6231 E ,2706-6223 P
Vol.11 No.4 (2025)

Phenotypic and Genotypic Detection of Metallo β-lactamases in Carbapenem Resistant *Klebsiella pneumoniae* Isolated from Various Clinical Specimen

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Abstract

The genus *Klebsiella is* the pathogenic species for humans, which are linked to numerous ailments, including pneumonia, bacteremia, and urinary tract infections. *K. pneumoniae* is among the most extensively researched species since it is frequently isolate from hospitals and is of particular concern because of its increased multidrug resistance. *K. pneumoniae* is one of the Enterobacteriaceae that has developed numerous resistance mechanisms, among which metallo-beta-lactamases (MBLs) are a major concern MBLs belong to the carbapenem-hydrolyzing class of enzymes that inactivate these essential antimicrobials as well as other β-lactamase .One hundred forty three specimen were collected from the patients attending the Baghdad Medical City Hospital, during the period from March 2024 to October 2024. All Specimens were cultured on MacConkey agar. The bacterial isolates were identified based on their cultural, morphological, and biochemical properties. Followed by VITEK-2 system. The final identification results showed that 31 isolates were confirmed as *K. pneumoniae*.and showed high resistant (100%) to Ampicillin, piperacillin/tazobactam, Imipenem, amikacin, ceftazidime (96.77%) to cefazolin , cefoxitin ceftriaxone, Cefepime (93.55%) to Gentamicin (87.10%) to Ciprofloxacin, Levofloxacin, Trimethoprim/Sulfamethoxazol and (64.52%) to Nitrofurantion. Phenotypic detection for MBL production by using combined disk diffusion test with EDTA the results showed that 25(74.2%) isolates were positive isolates and 6 (25.8%) isolates were negative. This indicates a high prevalence of MBL production in isolated *K. pneumoniae*. The genotypic detection of MBL genes show that NDM and VIM genes were detected in all 31 isolates showing a100% prevalence. The IMP gene were not detected in any of the 31 isolates the high detection to The NDM and VIM genes showing the high prevalence of MBL in local isolated *K. pneumoniae.*

Key words: Metallo β-lactamases; Carbapenem; *Klebsiella pneumoniae*

Introduction

Klebsiella pneumoniae was initially discovered in 1882 by Carl Friedlaender and was originally recognized as Friedlander's bacillus before being grouped with the new genus Klebsiella [1] *K. pneumoniae* is a Gram-negative bacteria. It is rod-shaped and a facultative anaerobe. Biochemical

properties include lactose fermentation, catalase positivity, and cytochrome oxidase negativity. The bacterium produces a thick extracellular polysaccharide capsule, assisting it evading host innate immune responses [2]. The genus Klebsiella is one of the pathogenic for humans, responsible for different diseases ranging from pneumonia to bacteremia and urinary tract infections. *K. pneumoniae* has been the one of the most studied species because of its nosocomial distribution and its high level of multidrug resistance[3] .

The occurrence of carbapenem resistant *Klebsiella pneumoniae* represents an enormous challenge for clinical microbiology and infectious disease, mainly because of their ability to withstand the broad activity of carbapenem antibiotics[4]. *Klebsiella pneumoniae* is one of the Enterobacteriaceae that has developed numerous resistance mechanisms, among which metallo-beta-lactamases (MBLs) are a major concern MBLs belong to the carbapenem-hydrolyzing class of enzymes that inactivate these essential antimicrobials as well as other β-lactamase[5].

Rapid and accurate diagnosis of MBL-producing isolates according to the clinical outcome is critical to reduce therapeutic failure and apply efficient treatment. Resistant to carbapenems *Klebsiella pneumoniae* (CRKP) presents a significant clinical challenge because to its resistance capabilities to the great majority of beta-lactams through the expression of diverse beta-lactamases, mainly metallo-beta-lactamases. The Ambler class B beta-lactamases; MBLs are zinc-requiring enzymes that are capable of inactivating the carbapenem and other β-lactam antibiotics. The MBLs could effectively be responsible of the CRKP traits, most of which Enzymes comprise New Delhi metallo-beta-lactamase-1 (NDM), Verona integron-encoded metallo-beta-lactamase (VIM), and imipenemase (IMP). which could make bacteria crucially resistant [6]. These resistance genes play a major role in the dissemination of carbapenem-resistant *Klebsiella pneumoniae*, and represent a key challenge to infection control in the healthcare environment.

The significant incidence of carbapenem resistance among indigenous isolates, along with the reality that these antibiotics serve as the final option for managing severe infections caused by this pathogen necessitates investigating the underlying genetic basis of this resistance in Iraq.

Methods:

Patients and clinical specimens' collection

One hundred forty three specimen were collected from the patients attending the Educational Laboratories and Surgical Hospital in Medical City, Baghdad from (September 2024 to January 2025), including Collection of 70 urine, 25 wound swabs, 7 sputum and 41 blood specimens. There were both male and female of varying ages up to 60 years. The clinical sample collection and processing were conducted as standard directions.

Identification of *K.pneumoniae* Isolate

Each specimen was cultivated on blood agar and MacConkey agar, incubated aerobically for 24 hours at 37°C [7]. The primary means of identifying bacterial isolates were their color, and morphologic traits prior to biochemical identification techniques [8]. Definitive identification test of the isolates was achieved by VITEK-2 system. The last

identification results showed that 31 isolates were confirmed as *K. pneumoniae*.

Phenotypic Detection of Metallo Beta-Lactamases (MBLs)

For phenotypic MBL finding, we employed the Combined Disc Test. Test organisms standardised to a 0.5 McFarland standard and inoculated onto Mueller Hinton agar, we then positioned two 10µg imipenem discs 25mm apart; one of these discs was supplemented with 10µl of 750µg EDTA (Sigma Chemicals, St. Louis, MO). Following 24 hours of incubation at 37°C, we conducted a careful comparison of the inhibitory zones. A notable increase of ≥ 7mm in the zone diameter surrounding the imipenem + EDTA disc, in comparison to the imipenem disc alone, was interpreted as positive for MBL production. A crucial preliminary step involved testing EDTA in isolation on the isolates to ensure it didn't cause false positives by inhibiting growth itself [9].

The PCR technique steps:

1- DNA Extraction of *K. pneumoniae* isolates

The Three to five isolated pure and fresh transplant colonies of MacConkey plate were suspending in Eppendorf tube with 1 mL of sterilized distilled water, and then. Afterwards, the cells were heated (at 100 °C in a water bath for 20 minutes) to get rid of the DNA free of other organelles. The homogenates were then immediately stored after being kept on ice for ~30 min, and the remaining cellular organelles were collected via centrifugation at 8000 rpm for 10 minutes. The supernatant was subsequently utilized as a DNA template [10].

2- Purity Determination and Determination of DNA yield

DNA detection was performed using UV-Visible spectrophotometry, as described by Koetsier and Cantor (2019)[11].DNA purity and the presence of contaminants were assessed by the A260 / A280. A ratio of 1.8 or greater indicates a purity DNA sample, signifying minimal contamination from proteins and other impurities.

In order to assess the quality of samples for use in subsequent processes, the Fluorescence Method was used, in which A Quants Fluor meter was used to calculate the extracted DNA concentration. Two microliters of DNA were mixed with 198 microliters of diluted Quantifluor Dye. Following five minutes of room temperature incubation in a dark area, DNA concentration values were detected.

3- Agarose Gel Electrophoresis Preparation

1. Preparation of 1 Liter of TBE Buffer (1X): 100 mL of 10X TBE combined with 900 mL of distilled water

2. Agarose Gel Preparation: A 100 ml aliquot of 10 X TBE was transferred into a beaker. Subsequently, 2g of agarose was introduced into the buffer as necessary to formulate a 2% agarose gel. For the low-melting agarose gel, the same concentrations were used. The solution was heated to its boiling point using a microwave. Subsequently, the solution was permitted to cool. Subsequently, 5 µL of Ethidium Bromide solution or Red save was included.

3. Horizontal agarose gel casting: At one end of the casting tray, the comb was placed, and the gel was assembled inside. After tape-

sealing both edges, the agarose solution was poured into the gel tray and allowed to cool at room temperature for half an hour (if using low melting agarose, it was cooled in the refrigerator for more than half an hour). After carefully removing the comb, the gel was put back into the electrophoresis chamber. There was enough TBE electrophoresis buffer in the chamber to submerge the gel by 1-2 mm. 2µl of Blue/Orange 6x Load Dye was applied to each PCR sample. 10 µl of DNA Marker II was introduced into the first lane of the gel. Each gel lane received 10 µl of the sample. The electrophoresis tank's lid was attached. One side of the device was connected to the cathode, while the anode was attached to the opposite side. The process was conducted for 40 minutes at 50 volts. The DNA was visualized using a UV trans illuminator.

4-Polymerase Chain Reaction (PCR) analysis

The presence of metallo B-lactamases genes was detected by genetic analysis using the primers in Table 1 by using PCR technique[12]. A total volume of 20 µl was used for the PCR, which consisted of 10 µl of EeasyTaq PCR Super Mix (China), 2 µl of DNA, 1 µl of each primer, and 6 µl of nuclease-free water. The reaction was performed under the best PCR conditions for each gene as show in table 2;3. The reference procedure was followed when setting up the amplification parameters for each primer kit in the heat cycler, and, as shown below, the polymerase chain reaction (PCR) tests were carried out in a 20 µl volume.

Table 1: The oligonucleotide primer sequences used in PCR amplification

Primer Name	Primer Sequence	annealing.T	Product size (bp)	References
IMP	**F** TGAGCAAGTTATCTGTATTC **R** TTAGTTGCTTGGTTTTGATG	61	740	(12)
VIM	**F** TTGGTCTACATGACCGCGTCT **R** TTTGACAACGTTCGCTGTGT	52	280	(12)
NDM	**F** ATTGCCCAATATTATGCACCC **R**GGAATGGCTCATCACGATCAT	58	748	(12)

Table 2: Reaction Setup and Thermal Cycling Protocol

Master mix components	Volume
PCR Master Mix	10
Primer F（10 μM）	1
Primer R（10 μM）	1
DNA	2
ddH2O	6
Total volume	20μl

Table 3: PCR Program

Primer	Denaturation	Annealing	Extension	Cycle number
NDM	94 C˚/20 sec	58 C˚/20 sec	72 C˚/20 sec	40
VIM	94 C˚/20 sec	52 C˚/20 sec	72 C˚/20 sec	40
IMP	94 C˚/20 sec	52 C˚/20 sec	72 C˚/20 sec	40

Results and Discussion

Isolation and identification of bacterial Isolates

One hundred forty three clinical specimen were collected from the patients attending the Baghdad Medical City Hospital, during the period from March 2024 to October 2024, as shown in Table 4. Specimens were cultured on MacConkey's agar. The bacterial isolates were recognized by their morphological characteristics and color. Morphological characters on MacConkey agar Colonies had pink colouration[8]. The stained smear was examined microscopically after Gram stain. Further characterization was done by oxidase and catralase tests, those which were negative for the oxidase test [13]. and positive for catalase test were included to next identification step[14]. Specimen were submitted to the next identification step, which was performed by the VITEK-2 system as a definitive identification tool**.** The result of these stages confirmed the identification of 60 *K. pneumoniae* isolates and 31 carbapenem-resistant *K. pneumoniae*, as shown in Table 4. The present study's

experiments encompassed these 31 isolates, as shown in Figure (1).

Table 4: Prevalence of Carbapenem-Resistant *K. pneumoniae* by Sample Type

Sample type	**No. of sample**	**No. of *K. pneumoniae* Isolate**	**No. of carbapenem-resistant *K. pneumoniae* isolates N (%)**
Blood	41	25	18 (72%)
Urine	70	20	7 (35%)
Wound swab	25	9	1 (11.1%)
Sputum	7	6	5 (83%)
Total	143	60	31 (51.6%)

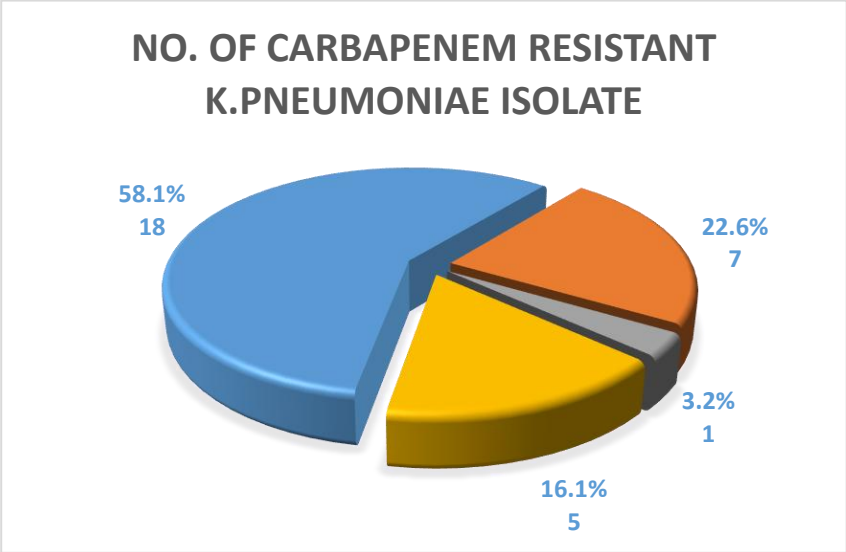

Figure 1. Distribution of Carbapenem-Resistant *Klebsiella pneumoniae* Isolates among Various Clinical Specimens

Table 4 and Figure 1 illustrate the distribution and prevalence of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates among various clinical specimen

types. Among the 143 clinical samples analyzed, urine was the most frequently collected specimen (70), followed by blood (41), wound swabs (25), and sputum (7). Notably, *K. pneumoniae* was isolated in 60 cases, with the highest proportion of carbapenem-resistant isolates observed in sputum samples (83%), despite this group having the smallest number of total samples. This suggests a significant burden of resistance in respiratory infections. Blood samples also showed a considerable carbapenem resistance rate of 72%, indicating a serious concern for bloodstream infections. Conversely, wound swabs and urine samples demonstrated relatively lower carbapenem resistance rates at 11.1% and 35 %, respectively.

The overall prevalence of carbapenem resistance in *K. pneumoniae* isolates was 51.6%, aligning with global trends of rising resistance among Enterobacteriaceae. Several studies have reported comparable resistance patterns. For example, a study in Egypt reported carbapenem resistance in 13 isolates of *K. pneumoniae* (16) isolates from hospitalized patients, which is consistent with the current findings [15]. Data from India indicated higher resistance rates in sputum (60%) and blood samples (40%), supporting the hypothesis that invasive infections are more likely to be caused by multidrug-resistant strains [16]. This pattern underscores the critical need for enhanced infection control and stewardship efforts.

These results highlight the heterogeneity of resistance across different specimen types and suggest that sample origin may serve as a predictor for carbapenem resistance in *K. pneumoniae*. Clinicians and microbiologists should consider this variation when selecting empirical treatment options and when designing surveillance programs. Figure 2 illustrates the distributions of Carbapenem-Resistant Klebsiella pneumoniae isolates (31) across different age groups**.**

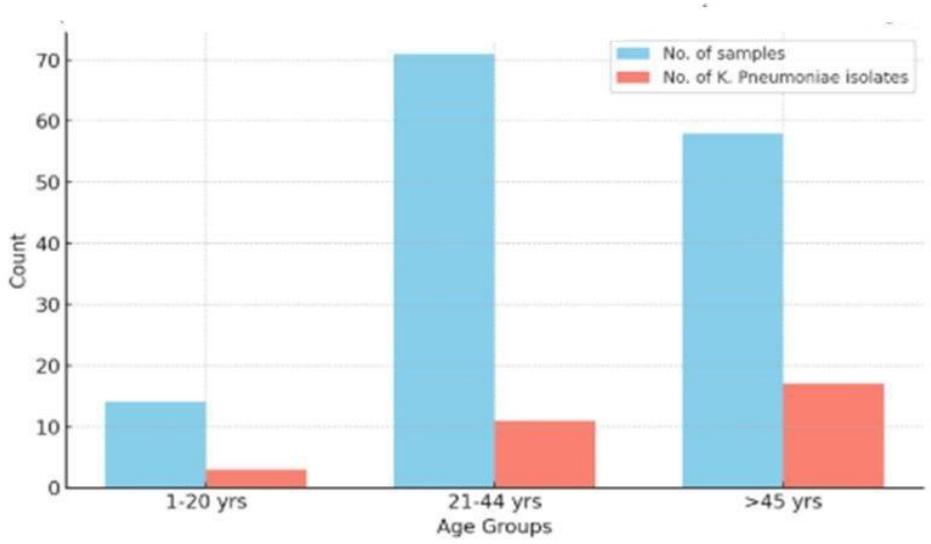

Figure 2. Distribution of *K.Pneumoniae* Isolates According to Age Groups

The age of the study subjects was between less than one year to 60 years.The age group greater than 45 years had the highest sum total of *K. pneumoniae* isolates 17 (54.83%) although the sample size is less compared to the second age group that is 21-44 years (11 isolates (35.48%). Generation of isolates The category with the least number of isolates in the youngest age group (1-20) years, 3(9.67%) a significant association between age and infection. These results suggest that *K. pneumoniae* infections are more frequent in older individuals, possibly due to immunosenescence, chronic illnesses, or increased healthcare exposure, as previously reported [17].

Multidrug Resistance in *Klebsiella pneumoniae*

Based on the VITEK-2 system results, which utilized five different groups of antibiotics, including Beta-lactams (Ampicillin, piperacillin/tazobactam, Imipenem, Cefazolin, Cefoxitin Ceftriaxone, Cefepime, and Ceftazidime), Aminoglycosides (Amikacin, Gentamicin), Fluoroquinolones (Ciprofloxacin, Levofloxacin), Nitrofurans (Nitrofurantoin) and Folate Antagonists (Trimethoprim/Sulfamethoxazol).

Subsequent to the exclusion of carbapenem-susceptible isolates, the resistance rates of carbapenem-resistant isolates were as follows: (100%) to Ampicillin, piperacillin/tazobactam, Imipenem, Amikacin, ceftazidime, (96.77%) to cefazolin, cefoxitin ceftriaxone, and Cefepime, (93.55%) to Gentamicin, (87.10%) to Ciprofloxacin, Levofloxacin, Trimethoprim\Sulfamethoxazol and (64.52%) to Nitrofurantion.

Multidrug-resistant trait (MDR) is characterized by resistance to at least one agent across three or more antimicrobial categories [18].

As shown in Table (8), we notice that all isolates were multidrug-resistant since all of them showed resistance to a minimum of one agent in three distinct antibiotic categories of the five categories involved in the study.

This data indicates a notable and simultaneous correlation between resistance to fluoroquinolones and nitrofurans observed in antibiotic susceptibility tests and patterns of multidrug-resistant profiles**.** This could be interpreted as the widespread dissemination of plasmid-encoded resistance genes among resistant isolates, or alternatively, that resistance to both agents is conferred by the same efflux pumps [19].

Phenotypic detection of Metallo Beta-Lactamase (MBL)

A metallo-β-lactamase (MBL) is distinguished by the presence of Zn2+ in its active site. Class B β-lactamases belong to the Bush-Jacoby functional group and facilitate the hydrolysis of carbapenems. In the presence of a chelating agent such as ethylenediaminetetraacetic acid (EDTA), the metal in the enzyme's active site prevents enzymatic activity [20].

Detection of Metallo Beta-Lactamase (MBL) via the EDTA combined disc assay for all *Klebsiella pneumoniae* isolates. Table (5) and figure (3) shows the results of MBL test for *Klebsiella pneumonia isolates* as MBL Positive isolates where these isolates showed significant increases in zone

diameter (≥7 mm) with EDTA and indicates the presence of MBL-producing *K. pneumoniae*.These strains can be resistant to carbapenems and are often multidrug-resistant. Whereas MBL Negative Isolates showed <7 mm difference in zone size.Indicates no MBL enzyme activity [9].

Table 5: The EDTA Combined Disk Test results of *K. pneumoniae Isolates* in the current study

K. pneumoniae isolate phenotype	Frequency (%)
MBL Positive isolates	25(74.2%)
Negative Isolates	6(25.8%)
Total	31

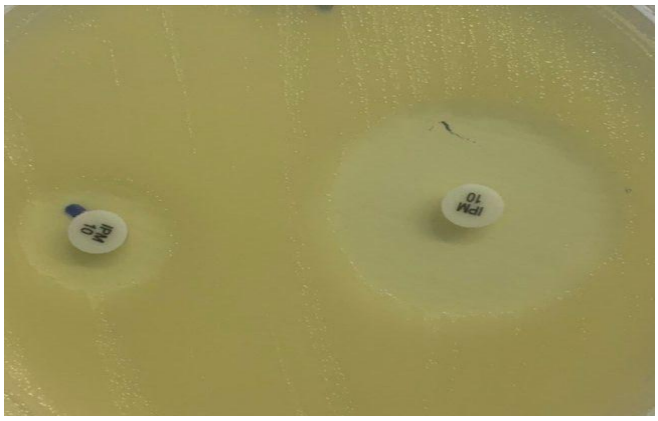

Figure 3. A positive result of Imipenem EDTA Combined Disk test for *Klebsiella pneumonia*

Table 5 and Figure 3 show that 74.2% (25/31) of carbapenem-resistant *K. pneumoniae* isolates were positive for Metallo-β-lactamase (MBL) production, while 25.8% were negative.

Previous studies have extensively employed phenotypic methods involving the use of combined disk diffusion test with EDTA for the detection of metallo-beta-lactamase (MBL) production in *Klebsiella pneumoniae* and other Gram-negative bacteria. In Egypt, the research of Khalifa *et al*. (2024)[21] reported that 36 (80%) out of *K. pneumoniae* isolates were MBL producers reflecting that such enzymes were also the most prevalent type of beta-lactamase associated with resistance, followed by ESBLs.

In line with the high phenotypic detection rates, researches carried out in India by Kaur *et al*. (2023)[23]who observed that 29 (82.85 %) of 35 Carbapenem Resistant K. *pneumoniae* as phenotypically positive as MBL producers by CDT.

And this indicating a dominant role of MBLs in carbapenem resistance among the isolates.

Genotypic detection of Metallo Beta-Lactamase (MBL) genes

MBLs are zinc-requiring enzymes that are capable of inactivating the carbapenem and other β-lactam antibiotics. The MBLs could effectively be responsible for the CRKP traits, most of which Enzymes comprise New Delhi metallo-beta-lactamase-1 (NDM), Verona integron-encoded metallo-beta-lactamase (VIM), and imipenemase (IMP). The MBL gene can reside on an integron, transposon, plasmid, chromosome, or various other genetic molecules, which could make bacteria crucially resistant [6].

The (NDM, VIM, IMP) MBL genes were identified at frequency among the carbapenem-resistant *K.Pneumoniae* analyzed in the PCR assay. Among the 31 isolates, the genotypic detection of MBL genes showed the NDM and VIM genes were found in all 31 isolates, showing a (100%) prevalence, and the IMP gene was not detected in any of the 31 isolates as show in table (6) and Figure (4)

Table 6: Distribution of Metallo Beta-Lactamase genes among carbapenem resistant *K.Pneumoniae* Isolate

MBL Genes	Number of positive Isolates	Percentage (%)
IMP	0	0.0
NDM	31	100
VIM	31	100

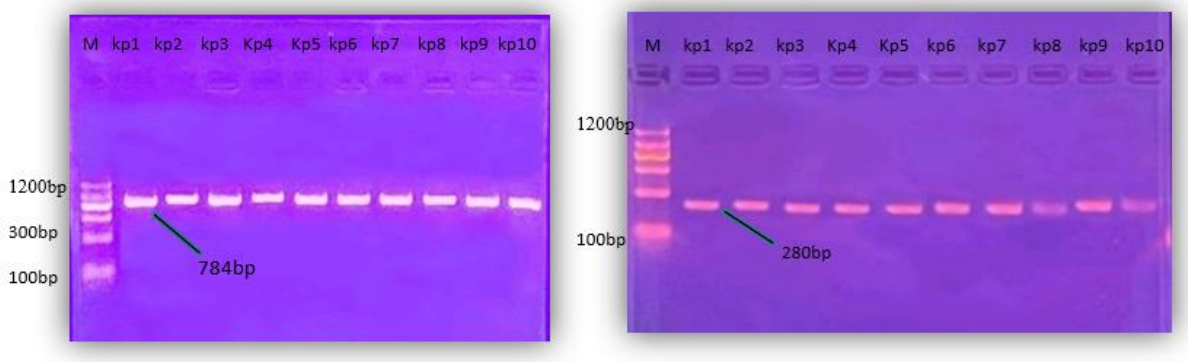

Figure 4. PCR product for NDM (784bp) and VIM (280bp) genes was analysed by gel electrophoresis (2% agarose gel, 50 V, 40 minutes) and visualised under UV light after ethidium bromide staini

The molecular studies also show substantial differences in the prevalence of MBL genes determined in different geographical areas. For example,a study by Ma *et al* (2024)[23],

conducted in China the ratio is as follows blaNDM and blaIMP (2.78%). In the same way, Taha *et al*. (2023)[24] reveled that percentages recording blaVIM, blaIMP and blaNDM were (15%),(7.5%) ,(3.8%) respectively . wall in Ghana a Study conducted by Dwomoh *et al*. (2022)[25] has also published different findings: blaNDM in 20%, but blaVIM, blaIMP were not found.

Our Data aligned with another native study conducted by Aboud (2024)[26] in Diyala, (9/11 isolates 81.81%) were blaNDM- 1, blaNDM-2 and blaVIM genes positive, and none of the isolates were found to be positive for blaIMP. Other native a study by [27] showed 11 isolates of *K. pneumoniae* (100%) were verified to possess the blaVIM gene, but no expression of the blaIMP gene was identified, in next year a study by[28] observed Six (85.71%) of the clinical isolates were positive for VIM, NDM, and OXA genes, while testing negative for IMP and KPC genes,

dissimilarly to a study in 2022 which showed that rates were for NDM (52%), and IMP (5.2%)[29], Studies conducted by [30] found that the NDM gene was identified in 19 out of 24 isolates, while the blaOXA-48 gene was identified in 5 out of 24 isolates. The existence of bla NDM denotes an alarmingly elevated prevalence of MBL in the *Klebsiella pneumoniae* strain.

A Clear correlation exists between the phenotype and genotype for MBL production as show in table (7) this refers to that, via the EDTA combined disc assay is an effective approach for phenotypic detection of MBLs.

Finally, the high frequency of blaNDM and blaVIM genes found in the present study may explain the statement that they are most common gene of metallo beta-lactamases prominently linked to carbapenem resistance in *K. pneumoniae* in this region.

Conclusion.

The detection of Metallo-beta-lactamases in carbapenem-resistant *Klebsiella pneumoniae* is of paramount importance in order to face the significant public health risk associated with the increase and spread of multidrug-resistant agents. The increasing incidence of carbapenem-resistant microorganisms worldwide has highlighted the importance of the recognition of Metallo-beta-lactamases (MBLs) for clinical microbiology and epidemiology. Appreciation of the role of these enzymes as resistance promoting pathways provides the basis for a more rational approach to diagnostic assessment and therapy choice. Quick and accurate detection of MBL-producing strains may lead to further treatment and prevent.

Table (7) The Profile of Phenotypic and genotypic detection of Metallo Beta-Lactamase (MBLs) in *Klebsiella pneumoniae*

Isolate ID	MBL phenotype	IMP gene	VIM gene	NDM gene
Kp1	✓	-	+	+
Kp2		-	+	+
Kp3	✓	-	+	+
Kp4	✓	-	+	+
Kp5		-	+	+
Kp6	✓	-	+	+
Kp7		-	+	+
Kp8	✓	-	+	+
Kp9	✓	-	+	+
Kp10		-	+	+
Kp11	✓	-	+	+
Kp12	✓	-	+	+
Kp13	✓	-	+	+
Kp14	✓	-	+	+
Kp15	✓	-	+	+
Kp16	✓	-	+	+
Kp17	✓	-	+	+
Kp18	✓	-	+	+
Kp19	✓	-	+	+
Kp20	✓	-	+	+
Kp21	✓	-	+	+
Kp22	✓	-	+	+
Kp23	✓	-	+	+
Kp24		-	+	+
Kp25		-	+	+
Kp26	✓	-	+	+
Kp27	✓	-	+	+
Kp28	✓	-	+	+
Kp29	✓	-	+	+
Kp30	✓	-	+	+
Kp31	✓	-	+	+

Table 8: Antimicrobial Agent Susceptibility Profile of Carbapenem-Resistant *Klebsiella pneumoniae* Isolates

Isolate ID	AM	PTZ	KF	FOX	CAZ	CRO	FEP	IMP	AK	GM	CIP	LEV	FTN	SXT
Kp1	R	R	S	S	R	S	R	R	R	R	S	S	S	R
Kp2	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp3	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp4	R	R	R	R	R	R	R	R	R	R	R	R	R	S
Kp5	R	R	R	R	R	R	R	R	R	R	R	R	R	S
Kp6	R	R	R	R	R	R	R	R	R	S	R	R	R	R
Kp7	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp8	R	R	R	R	R	R	R	R	R	R	R	R	R	S
Kp9	R	R	R	R	R	R	R	R	R	R	S	S	S	R
Kp10	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp11	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp12	R	R	R	R	R	R	R	R	R	R	S	S	S	R
Kp13	R	R	R	R	R	R	R	R	R	R	S	S	S	R
Kp14	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp15	R	R	R	R	R	R	R	R	R	R	R	R	I	R
Kp16	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp17	R	R	R	R	R	R	R	R	R	R	R	R	I	R
Kp18	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp19	R	R	R	R	R	R	R	R	R	R	R	R	I	R
Kp20	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp21	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp22	R	R	R	R	R	R	R	R	R	R	R	R	S	R
Kp23	R	R	R	R	R	R	R	R	R	R	R	R	I	R
Kp24	R	R	R	R	R	R	R	R	R	R	R	R	I	R
Kp25	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp26	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp27	R	R	R	R	R	R	R	R	R	R	R	R	I	R
Kp28	R	R	R	R	R	R	S	R	R	I	R	R	I	R
Kp29	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp30	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Kp31	R	R	R	R	R	R	R	R	R	R	R	R	R	S

AM: Ampicillin; PTZ: piperacillin/tazobactam; KF: cefazolin; FOX: cefoxitin; CAZ: ceftazidime; CRO: ceftriaxone FEP: Cefepime; IMP: Imipenem; AK: amikacin; GM: Gentamicin; CIP: Ciprofloxacin; LEV: Levofloxacin; TX: Trimethoprim\Sulfamethoxazol; FTN: Nitrofurantion. R: Resistant, S: Sensitive I: Intermediate

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التحري المظهري والوراثي عن انزيمات البيتلاكتام المعدنية في عزلات بكتريا الكلبسيلا الرئوية المقاومة للكاربابنيم المعزولة من عينات سريرية مختلفة

الخلاصة

تعد بكتيريا الكليبسيلا (Klebsiella) من الأنواع الممرضة للإنسان، إذ ترتبط بالعديد من الأمراض، بما في ذلك الالتهاب الرئوي، وتجرثم الدم، والتهابات المسالك البولية. وتُعد الكليبسيلا الرئوية (*Klebsiella pneumoniae*) من أكثر الأنواع المدروسة على نطاق واسع، نظرًا لعزلها المتكرر من المستشفيات ولأهميتها الخاصة بسبب ارتفاع مستوى مقاومتها المتعددة للمضادات الحيوية.تُعتبر *K. pneumoniae* أحد أفراد عائلة المعوية التي طورت العديد من آليات المقاومة، ومن أبرزها إنزيمات البيتا-لاكتام المعدنية .(Metallo-β-lactamases, MBLs) وتنتمي هذه الإنزيمات إلى فئة الإنزيمات القادرة على تحليل الكاربابينيمات، مما يؤدي إلى تعطيل فعالية هذه المضادات الحيوية المهمة بالإضافة إلى العديد من مضادات البيتا-لاكتام الأخرى. جمعت 143عينة سريرية من المرضى المراجعين لـمستشفى مدينة بغداد الطبية خلال الفترة الممتدة من مارس 2024 إلى أكتوبر 2024. زُرعت جميع العينات على وسط MacConkey agar، وتم تشخيص العزلات البكتيرية اعتمادًا على خصائصها الزرعية والمظهرية والكيميائية الحيوية، ثم تأكيد التشخيص باستخدام جهاز VITEK 2.

أظهرت نتائج التشخيص النهائية أن 31عزلة تم تأكيدها على أنها .*K. pneumoniae* وأظهرت هذه العزلات مقاومة مرتفعة جدًا للمضادات الحيوية، حيث بلغت نسبة المقاومة 100%لكل من الأمبيسيلين، والبيبيراسيلين/تازوباكتام ، والإيميبينيم، والأميكاسين، وبلغت 96.77%للسيفازولين ، والسيفوكسيتين، والسيفترياكسون، والسيفيبيم، 93.55% للجنتتامايسين ، و 87.10% للسيبروفلوكساسين ، والليفوفلوكساسين، والتريميثوبريم/سلفاميثوكسازول ، بينما بلغت 64.52% للنيتروفورانتوين ..كما تم الكشف الظاهري عن إنتاج إنزيمات MBL باستخدام اختبار الانتشار بالأقراص المزدوجة مع EDTA ، وأظهرت النتائج أن 25عزلة (74.2%) كانت موجبة لإنتاج هذه الإنزيمات، في حين كانت 6عزلات (25.8%) سالبة. وتشير هذه النتائج إلى الانتشار المرتفع لإنتاج إنزيمات MBL بين عزلات *K. pneumoniae* المعزولة.

أما الكشف الوراثي لجينات MBL فقد أظهر وجود جيني NDMو VIMفي جميع العزلات البالغ عددها 31 عزلة، بنسبة انتشار بلغت .100%في المقابل، لم يتم الكشف عن جين IMPفي أي من العزلات. ويؤكد الانتشار المرتفع لجيني NDM و VIMعلى شيوع إنزيمات MBL بشكل كبير بين عزلات *K. pneumoniae* المحلية المعزولة.