The Prevalence of Integron Class I and Class II Among Carbapenem Resistance Klebsiella Pneumonia: A Cross-Sectional Study

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Abstract: The global frequency of Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) continues to rise, causing the World Health Organization to declare it a critical threat to the health of public. CRKP was regarded as a challenging organism to treat due to restricted therapy alternatives. This study aimed to determine the antibiotics resistance patterns and the prevalence of Integron I, II, and their associated genes in *K. pneumoniae* isolated from various clinical specimens. Overall, 300 clinical samples obtained from (blood, urine, sputum, burns, and wounds), fifty (16.66%) isolates were identified as *K.pneumonia*. Out of 50 isolates. Antimicrobial sensitivity test were performed for all Fifty isolates of *K. pneumonia*,16(32%) from isolates have been resistance to carbapenem. The prevalence of *K. pneumoniae* isolates by origin was 11 (22%) from urine. 15(30%) wound, 10(20%) from sputum, 11(22%) from was burns and only 3(6%) blood, The isolates were examined for class I and II integrons as well as carbapeneme resistance genes using PCR. Antibiotics sensitivity indicated all of the isolates 50(100%) were resistance to piperacillin/tazobactam,49(98%) resistance to cefoxitin, and the lowest resistance against to chloramphenicol. Amplification of PCR products revealed that 12 (75%) of the isolates exhibited class I (*intl1*) integrons, whereas 8 (50%) displayed class II (*intl2*) integrons, *blaIMP-1* was the most prevalent carbapenem resistance gene 10 (62.5%), followed by bla_{NDM} 6(37.5%), bla_{OXA-48} 5(31.25%), *blaIMP-2* 3(18.75%), *blaVIM-2* 2(12.5%) and *blaKPC-1* 1(6.25%). 8 hyper-virulence strains tested positively to *blaOXA-48*, while 2 had bla_{NDM} genes.

1 INTRODUCTION

Klebsiella pneumoniae (K. pneumoniae) constitutes a non-motile, encapsulated gram-negative bacillus measuring around 1–2 μm in length and was classified as a facultative anaerobic [1]. It becomes a prevalent etiological agent of urinary tract, soft-tissues, with neurological infections, as well as endocarditis, and instances for serious bronchopneumonia, occasionally resulting in chronic constructive lesions and numerous pulmonary abscesses. Localized infections frequently result in bacteremia [1].Multidrug-Resistant Enterobacterales, particularly *K. pneumoniae*, constitute a major source of health carly associated infections, resulting in increased mortality with morbidity along with rising medical expenses [2]. Carbapenems have extensive anti-bacterial efficacy and include a distinctive structure characterized by a carbapenem linked to the ring of β-lactam, that provides protection against most β-lactamases, including metallo-β-lactamase (MBL) and extended-spectrum β-lactamases (ESBLs) [3]. Thus, carbapenems are regarded as among the most dependable agents for managing infections of bacteria, and the establishment and proliferation increasing resistant to these antibiotics represent a significant public health issue [4]. CRKP a constituent of the carbapenem-resistant Enterobacterales (CRE) family [5]. The carbapenemase enzymes of *K. pneumoniae* are able to withstand the majority of antibiotics that contain β-lactam rings, includes carbapenems, which makes these medications resistant to them [6]. Class A *K. pneumoniae* carbapenemase (*KPC*), class B Verona integron metallo-betalactamases (*VIM*), class D oxacillinase-48 (*OXA-48*), and class A Imipenemase (*IMP*) are commonly found in high concentrations in patients with serious infections in hospitals [7]. The illness has expanded rapidly across China since the initial bla_{NDM-1} positive *K. pneumoniae* isolates were found in Nanchang in 2013. Some regions have had epidemics of *K. pneumoniae* that produce *NDM-1* [8]. These regions include Shanghai, Hunan, and Yunnan. The collection of around 400 enzymes

with unique genetic makeup is called Class-D β-lactamase, OXA-type enzymes, or oxacillinases [9]. Carbapenemase activity is confined to a small subset of the class-D OXA family. Common ones include OXA-48, OXA-23, and OXA-40; variants on this theme include OXA-232, OXA-162, and OXA-181 [9]. The majority of clinical gram-negative bacteria, includes *Yersinia, Shigella, Klebsiella, Salmonella*, and *Escherichia coli*, have class 1 integrons due to their interaction with transposons, which are frequently found inside conjugative plasmids [10]. But healthy individuals of any age can get infected with hvKp at more than one place. The pathogenicity and capacity to acquire mobile genetic elements, such as integrons and transposons, have been investigated in several investigations. Bacteria can exchange resistance gene cassettes with one another by the use of integrons, which are strong mobile genetic components [11]. So far, five distinct types of integrons that encode antibiotic resistance have been discovered. Clinical isolates of gram negative bacteria, such as *Klebsiella pneumoniae*, most commonly include class I integrons (*intI*), while class II integrons have only somewhat been studied [12], [13]. Class 1 integrons possess variable gene cassettes regions that may occasionally be absent from the integron framework, flanked by two exceptionally preserved sequences: the 5′-conserved segment (5′-CS) and the 3′-conserved segment (3′-CS) [14]. Genes cassettes constitute promoterless, changeable segments of as located among the attC and attI regions which encoding resistance to antibiotics characteristics. Their expression was regulated by the integron promoter (Pc), that, in the case of class 1 integrons, depends on the 5′-CS. Numerous cassettes array were documented, predominantly consisting of two or three gene cassettes [15]. Although many investigations have recorded the frequency of integrons in cKp obtained from clinical samples in Iraq, there's a lack of study concerning the frequency for Class 1, 2, and 3 integrons in hvKp isolates from nosocomial infections in our region. The present investigation sought to determine the prevalence of class 1 and 2 integrons in clinical cKp isolates across Diyala province, Iraq [16].

2 MATERIALS AND METHODS

2.1 Research Methodology with Participants

Between July 2024 and February 2025, researchers in Iraq's Diyala region conducted a cross-sectional study at two hospitals: Baquba teaching hospital and Al-batul hospital. We collected diverse samples from men and women patients of different ages, as well as from wounds, sputum, blood, and burn swabs.

2.2 Isolates of Bacteria

From different clinical samples, 50 *K. pneumoniae* isolates were obtained. The ornithine decarboxylase (OD) test, urease test, methyl red/Voges-Proskauer (MR/VP) test, and citrate consumption test were some of the traditional microbiological and biochemical techniques used to identify bacterial isolates. Afterwards, verified with the Vitek-2 apparatus [17]. The bacterial isolates were stored at -70°C in TSB with 20% glycerol (HiMedia, Indian) before they were needed.

2.3 Antibiotics Sensitivity Test

The antibiotic sensitivity for *K. pneumoniae* isolates has been evaluated utilising the disc diffusion technique, as per the requirements set by the Clinical and Laboratory requirements Institute (CLSI 2023). This investigation utilized twenty antimicrobial discs provided by Hi-Media Pvt. Ltd., Mumbai, Indian. amikacin (AK 30 μg), tetracyclin (TE 10 μg), Aztreonam (AT 30 μg), Gentamicin (GEN 10 μg), Ciprofloxacin(CIP 10 μg), norfloxacin (NOR 30 μg), imipenem (IMP 10 μg), streptomycin (HLS 30 μg), Ofloxacin (OEX 5 μg), and Meropenem (MEM 10 μg),,cefepime (CPM5 μg),cefoxitin (CX 10 μg), Sulfametoxazol/Trimethoprime(STX 5 μg) Ampicilin (AMP 10 μg), Amoxicillin-clavulanic acid(AMC 10 μg),Chloramphenicol (C), Doxycyclin (DOX), Fosfomicin (FOS 10 μg), Nitrofurantoin (NIT10 μg), Piperacillin/Tazobactam (PIT 100/10 μg). The verified *K. pneumoniae* samples have been preserved at −70°C in tryptic soy broth (TSB) with 20% glycerol and underwent molecular detection.

2.4 ESBL Phenotypic Confirmation

The original strategy utilized was the combination disc method. In accordance with CLSI recommendations (2023), the following were used on Mueller-Hinton agar (MHA): ceftazidime (30 µg), ceftazidime clavulanate (30/10 µg), cefotaxime (30 µg), and cefotaxime clavulanate (30/10 µg). Incubation at 37 °C was maintained for 16–18 hours on the plates. The use of marker Cephalosporins combined with Clavulanate, rather than marker Cephalosporins alone, resulted in a 5-mm rise in zone diameter for the identification of ESBL

producers [18], [19]. The combination disc technique was used with and without clavulanic acid (CLA; 10 µg), using CTX (30 µg) and CAZ (30 µg). We compared the diameter of the resultant inhibitory zone [20]. When there was a variance of 5 mm or more in zone diameters among the individual and combined discs, the test was deemed positive.

2.5 Detection of Metallo-β-Lactamase Activity Using Phenotypic Method

All isolates that were found to be resistant to carbapenem were tested,imipenem (the zone dimeter ≤19 mm according to CLSI, 2023 recommendations) was used by employing disc diffusion technique on MHA were testing for metallo-β-lactamase activity utilizing the imipenem+EDTA combination disc test as described in previous research [21] In short, a colony from an overnight growth of the test organism was placed on a MHA plate the suspension was ensured to be 0.5 McFarland, which is the equivalent of 1.5×10^8 CFU/ml of bacterial suspension density. A disc of imipenem (10 µg) was embedded into an inoculated plate, and a similar antibiotic disc but with EDTA was placed on the opposite side of the plate 5 mm apart. at 37°C the plates underwent incubation for 16 to 18 hrs. the inhibition zones of both discs were compared.

2.6 Sequencing for Primers

The primers employed in this investigation are enumerated in Table 1.

2.7 DNA Extraction

Bacterial DNA extraction is performed as the following [16] five millilitres from LB broth was utilized in order to cultivate the culture of bacteria, and then transferred one millilitre from that culture to Eppendorf tubes. Without touching the particle, the supernatant was removed after centrifuging the tubes for 3 minutes at 8000 rpm. Using the ABC DNA Isolation Kits, genomic DNA was isolated. The spectrophotometer (Nanodrop) was used to ascertain the quantity and quality of the DNA.

2.8 DNA Quantification

The concentration for the DNA extracted was detected using a Quantus Fluorometer to ensure the samples were of high quality for subsequent uses.
The following was mixed: 1 microlitre of DNA with 199 microlitres of diluted quantifluor dye. After incubating at ambient temperature for 5 minutes, the DNA concentration values were taken.

2.9 Molecular Identification for Resistance Factors

Table 1 lists the primers that are used for polymerase chain reaction. The reactions were conducted in accordance with Table 2 using a 25 µl final volume in a Techne TC-512 thermocycler (Germany). Following 60 minutes of running on a 1.0% agarose/TBE 0.5X (45 mM-Tris-borate, 1 mM-EDTA, pH = 8.0) gel stained with DNA safe dye, the PCR products were exposed to the UV trans-illuminator.

Table 1: Primer pair sequences utilized in this investigation.

Target gene	Direction	Primer sequences (5'→3′)	Amplicon size (bp)	Ref
BlaKPC-1	F	ATGTCACTGTATCGCCGTCT	538	[22]
	R	TTTTCAGAGCCTTACTGCCC		
BlaIMP-1	F	TGAGCAAGTTATCTGTATTC	740	[22]
	R	TTAGTTGCTTGGTTTTGATG		
BlaIMP-2	F	GGCAGTCGCCCTAAAACAAA	139	[22]
	R	TAGTTACTTGGCTGTGATGG		
BlaVIM-1	F	GATGGTGTTTGGTCGCATA	390	[22]
	R	CGAATGCGCAGCACCAG		
BlaNDM-1	F	GGTTTGGCGATCTGGTTTTC	621	[22]
	R	CGGAATGGCTCATCACGATC		
BlaOXA-48	F	TTGGTGGCATCGATTATCGG	744	[22]
	R	GAGCACTTCTTTTGTGATGGC		
INT1	F	CAGTGGACATAAGCCTGTTC	160	[16]
	R	CCCGAGGCATAGACTGTA		
INT2	F	CACGGATATGCGACAAAAAGGT	Variable	[16]
	R	GTAGCAAACGAGTGTGACGAAATG		

Table 2: Ideal Parameters for the Amplification of *qnr* genes via PCR.

Gene	Initial denaturation	Cycling condition			Final extension	Cycles
		denaturation	Annealing	Extension		
BlaKPC	94 ℃/3min	94 ℃/40 sec	53 ℃/40sec	72 ℃/40sec	72 ℃/2min	35
BlaIMP-1	94 ℃/3min	94 ℃/40 sec	53 ℃/40sec	72 ℃/40sec	72 ℃/2min	
BlaIMP-2	94 ℃/3min	94 ℃/40 sec	53 ℃/40sec	72 ℃/40sec	72 ℃/2min	
BlaVIM	94 ℃/3min	94 ℃/40 sec	60 ℃/40sec	72 ℃/40sec	72 ℃/2min	
BlaNDM	94 ℃/3min	94 ℃/40 sec	55 ℃/40sec	72 ℃/40sec	72 ℃/2min	
BlaOXA-48	94 ℃/3min	94 ℃/40 sec	55 ℃/40sec	72 ℃/40sec	72 ℃/2min	
INTI1	94 ℃/3min	94 ℃/40 sec	54 ℃/40sec	72 ℃/40sec	72 ℃/2min	30
INTI2	94 ℃/3min	94 ℃/40 sec	60 ℃/40sec	72 ℃/40sec	72 ℃/2min	

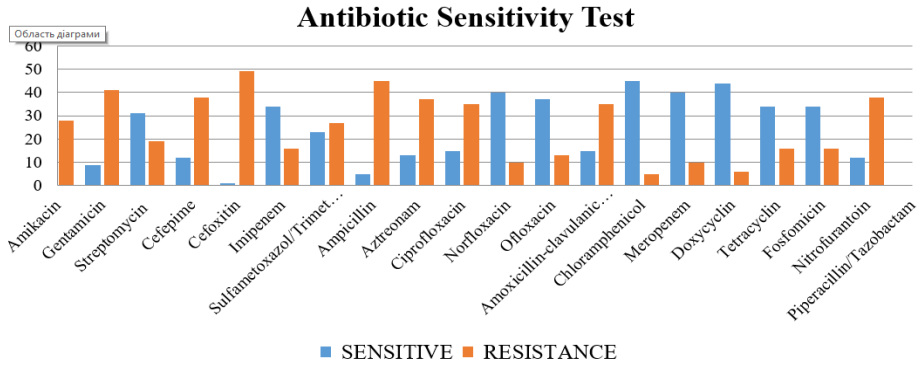

Figure 1: Antibiotic susceptibility of *K.pneumoniae* isolates (n= 50).

2.10 Electrophoresis on Agarose Gels

Utilizing a DNA ladder (100-1500 bp) provided by Promega (USA) as a size marker for the DNA, the products of PCR have been running on a 1% agarose gel stained with 0.5 µg/ml ethidium bromide in 1X TAE buffer. The electrophoresis was run for 80 minutes at 100 V. To observe the product of PCR products under 320 nm UV light, the UV-Transilluminater was utilized [23].

2.11 Statistics Assessment

The statistics study was conducted utilizing SPSS, version 25.0, a program developed by the IBM Companies in Chicago. Presentation of categories of variables was done using percentages and frequencies.

3 RESULTS AND DISCUSSION

3.1 Isolation and Identification

From a total 300 different sample have been obtained from Baqubah Teaching hospital and Al-Batul hospital in Diyala province, Iraq, were including (urine, wound, sputum, burn, and blood), 50 (16.66 %) *Klebsiella pneumonia*, Initial bacterial identification relied on biochemicals, microscopic, and culture testing. A Vitek 2-compact system [24] verified that. Based on Tab (3), the distribution of *K. pneumoniae* isolates by origin was as follows: 11(22%) from urine, 15(30%) from wounds, 10(20%) from sputum, 11(22%) from burns, and only 3(6%) from blood (Table 3).

Table 3: Distribution of *K. pneumoniae* based on source.

Sample type	Total *K. pneumonia* isolates (%)
Urine	11(22%)
Wound	15(30%)
Sputum	10(20%)
Burn	11(22%)
Blood	3(6%)
Total	50(100%)

3.2 Resistance to Anti-Microbials

The sensitivity test for twelve anti-biotics was assessed using the disc diffusion assay (Kirby-Bauer technique), as illustrated in Figure 1, in compliance with (CLSI) criteria (2023). A variety of antibacterial agents resistant activities were observed in the tested isolates. 50% of *K. pneumoniae* isolates (50/100)

were resistant to piperacillin/tazobactam, and nearly all of the other 49 (98%) were as well. While meropenem constitutes one of the more efficient antibiotics against *K. pneumoniae*, the isolates tested in this study shown moderate resistance to 10 of the medicines (50%) and tetracyclin as well (50%) to the same extent.

Every single 50%, or 100%, from *K. pneumoniae* isolates tested have been resistance to three or more for antibiotics tested. According to Table 4, the percentage of isolates resistant to different classes was as follows: 11 (22%) were resistance to 7 classes, 10 (20%) to 9 classes, 7 (14% to 14%) to 5 and 8 classes, 5 (10%) to 4 and 6 classes, 3 (6%) to 10 classes, and 1 (2% to 11) classes. The combination disc technique estimated that 66% of *K. pneumoniae* isolates produced ESBLs (33 isolates), whereas 20% of isolates had MBLs (ten isolates).

Table 4: Distribution of isolates resistant to different classes.

Number of classes	Number of isolates (%)
3 classes	1(2%)
4 classes	5(10%)
5 classes	7(14%)
6 classes	5 (10%)
7 classes	11 (22%)
8 classes	7(14%)
9 classes	10 (20%)
10 classes	3 (6%)
11 classes	1(2%)
Total	50

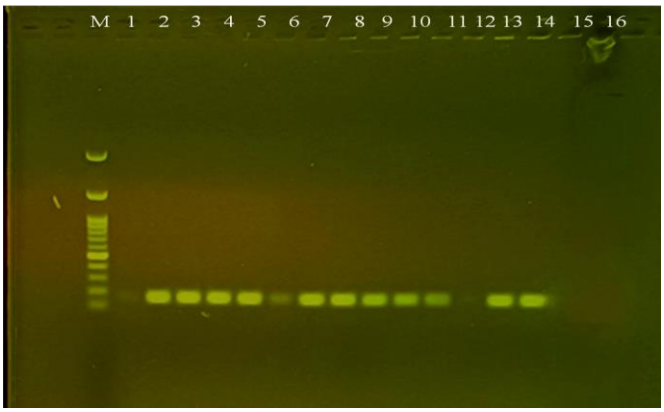

Figure 2: The gel electrophoresis for PCR results demonstrated *IntI* genes measuring 160 bp on 1% agarose at 100V for 80 minutes. Lane M: DNA ladder (100-1500 base pairs). Lanes 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13 represent Isolates positive for *K. pneumoniae* using PCR.

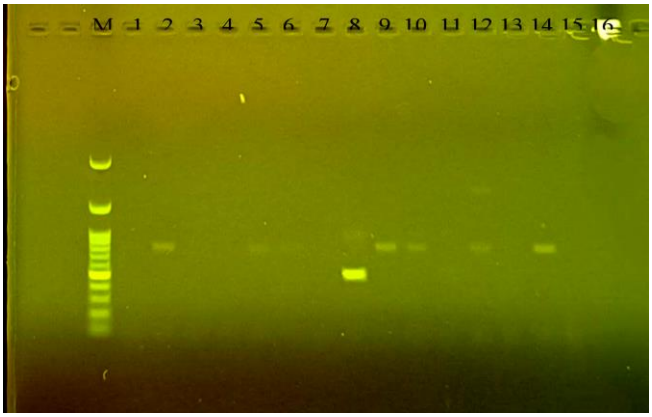

Figure 3: The electrophoresis gel of PCR results demonstrated *IntII* genes with variable size on 1% agarose at 100V for 80 minutes. Lane M: DNA ladder (100-1500 base pairs). Lanes 2, 5, 6, 8, 9, 10, 12, and 14 denote PCR positive isolates of *K. pneumonia*.

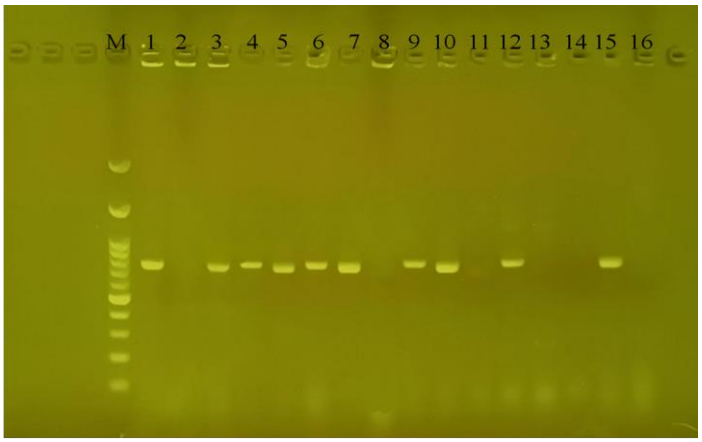

Figure 4: The gel electrophoresis for PCR results revealed the *blaIMP-1* gene at 790 bp on 1% agarose at 100V for 80 minutes. Lane M: DNA ladder (100-1500 base pairs). Lanes 1,3,4,5,6,7,9,10,12,15 represent *K. pneumoniae* PCR positive isolates.

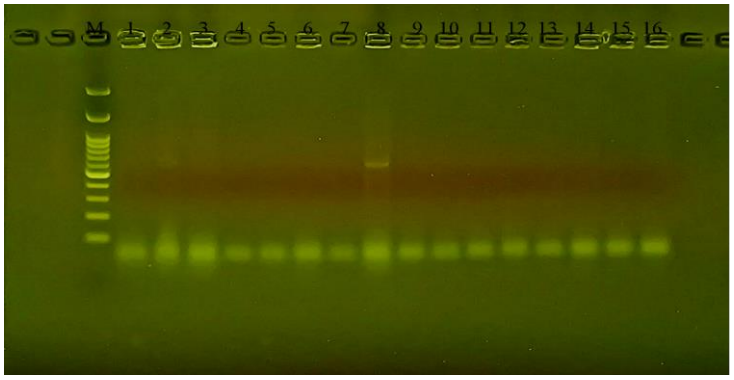

Figure 5: The electrophoresis gel for PCR results revealed the *blaKPC-1* gene at 538 bp on 1% agarose at 100V for 80 minutes. Lane M: DNA ladder (100-1500 base pairs).Lane 8 represent *K. pneumoniae* PCR positive isolate.

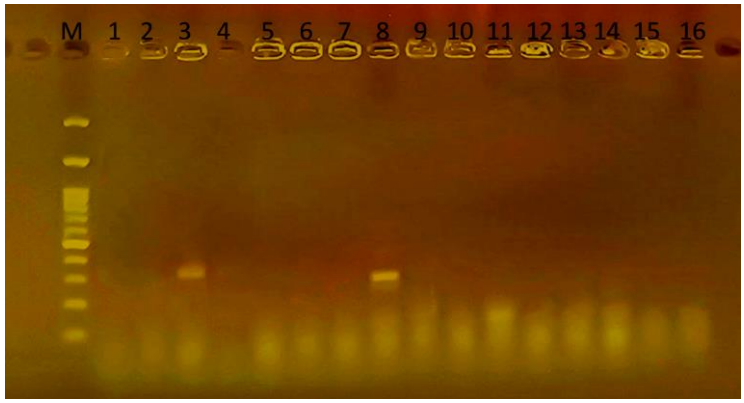

Figure 6: blaVIM-2 genes with 390 bp were visible on 1% agarose gel electrophoresed at 100 V for 80 minutes from PCR results. Gene ladder (100-1500 bp) is located in Lane M. Positive *K. pneumoniae* PCR isolates are seen in lanes 3, and 8.

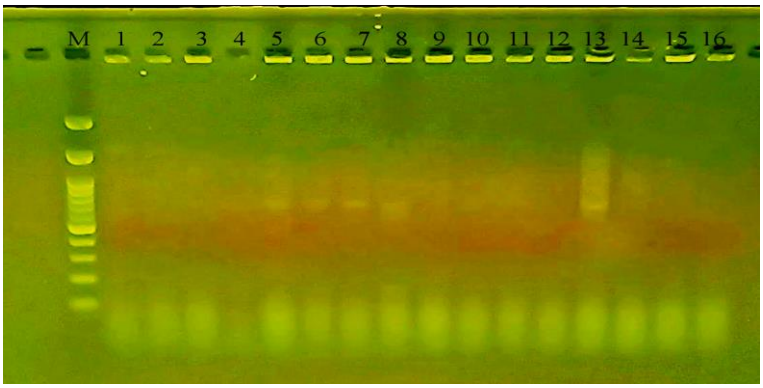

Figure 7: The gel electrophoresis of PCR results on 1% agarose at 100V/80 min revealed *blaOXA-48* genes 790 bp. Gene ladder (100-1500 bp) is located in Lane M. Positive *K. pneumoniae* PCR isolates are seen in lanes 5, 6, 7, 8, and 13.

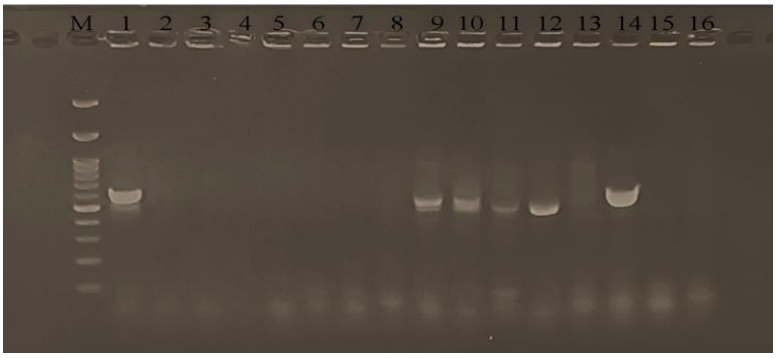

Figure 8. Electrophoresis for PCR products on 1% agarose at 100V for 80 mins revealed the *blaNDM-1* gene with 521 bp. Gene ladder (100-1500 bp) is located in Lane M. The *K. pneumoniae* PCR positive isolates are shown in lanes 1, 9, 10, 11, 12, and 14.

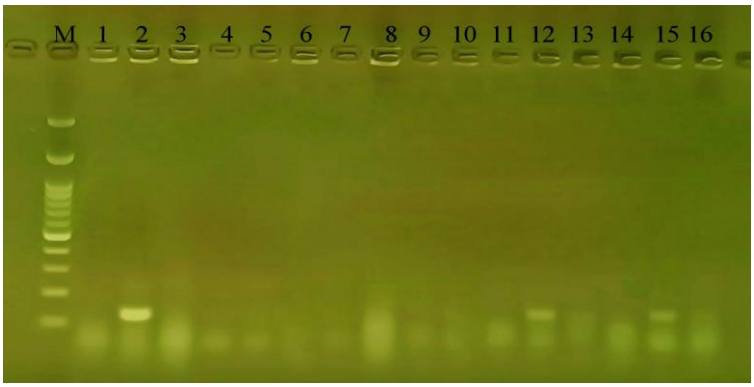

Figure 9: The gel electrophoresis for PCR results on 1% agarose at 100V/80 minutes revealed the *blaIMP-2* gene with139 bp. Gene ladder (100-1500 bp) is located in Lane M. Lanes 2, 12, and 15 show isolates that tested positive for *K. pneumoniae* by polymerase chain reaction.

Table 5: Prevalence of Integrones classI and class II and carbapenemase-encoding genes in total isolates of *K. pneumonia* (n=16).

Tested genes	Frequency of genes (%)
intl1	12 (75%)
intl2	8(50%)
bla$_{\text{IMP-1}}$	10(62.5%),
bla$_{\text{NDM-1}}$	6(37.5%),
bla$_{OXA-48}$	5(31.25%),
bla$_{\text{IMP-2}}$	3(18.75%)
bla$_{\text{VIM-2}}$	2(12.5%)
bla$_{\text{KPC-1}}$	1(6.25%)

Table 6: Distribution of genetic determinant and source of sample in total *Klebsiella pneumonia* isolates (n=16).

Isolates name	Sample source	Carbapenem resistant genes	Integrone
Kp1	Wound	*IMP-1, NDM-1*	classI
Kp2	Wound	*IMP-2*	classI and II
Kp3	Blood	*IMP-1,VIM-1*	classI
Kp4	Sputum	*IMP-1*	classI
Kp5	Wound	*IMP-1,OXA-48*	classI and II
Kp6	Wound	*IMP-1,OXA-48*	classI and II
Kp7	Wound	*IMP-1,OXA-48*	classI
Kp8	Sputum	*OXA-48,VIM-1,KPC*	classI and II
Kp9	Wound	*IMP-1,NDM-1*	classI and II
Kp10	burn	*IMP-1,NDM-1*	classI and II
Kp11	Sputum	*NDM-1*	classI
Kp12	Wound	*IMP-1,NDM-1, IMP-2*	class II
Kp13	Wound	*OXA-48*	classI
Kp14	Burn	*NDM-1*	classI and II
Kp15	Burn	*IMP-1,IMP-2*	-
Kp16	Blood	-	-

3.3 Integrones Class I and Class II and Carbapenemase-Encoding Genes Distribution

According to PCR amplification, out of 16 (32 %) of carbapenem resistant *K.pneumonia* isolates, 12 isolates (or 75% of the total) displayed the proportion of class I (*intl1*) and eight isolates (or 50% of the total) exhibited class II *(intl2*) Integrons. Of those, *blaIMP-1* was the most prevalent carbapenem-resistance gene, accounting for 62.5%. followed by *bla*$_{\text{NDM-1}}$ 6(37.5%), *bla*$_{OXA-48}$ 5(31.25%), *bla*$_{\text{IMP-2}}$ 3(18.75%), *bla*$_{\text{VIM-2}}$ 2(12.5%) and *bla*$_{\text{KPC-1}}$ 1(6.25%) Table 5, Table 6 and Figures 2 - 9.

Integrons constitute mobile genomic components that harbour significant genetic determinants for resistance to antibiotics. These components possess many classes and significantly contribute to the evolution of resistant to antibiotics in gramnegative bacteria. The MDR variant of *K. pneumoniae*, a prevalent nosocomial pathogen that frequently induces challenging infections worldwide, poses a significant public health threat [25]. The escalating resistance to antibiotics for bacteria, particularly *K. pneumoniae*, has resulted in significant challenges in the management of infectious illnesses. [26], [27]. Resistance genes to antibiotics can be disseminated, with integrons playing a crucial part in this process [28]. Integrons, genetic entities that harbour resistant genes to several antibiotics, were first characterized by Hall and Stokes [29]. The findings from this investigation indicated that 12 (75%) of multidrug-resistant *K. pneumoniae* tested positive for class I integron, this aligns with the findings by Firoozeh *et al.* [30] and additionally is consistent with the work of Mirkalantari *et al*. [31]. CRKP has been documented in the majority of Gulf Cooperation Council nations [32]. The frequency of *IntII* in our sample was 8 (50%). The integron PCR analysis findings from the study by Ahangarzadeh *et al.* (2012) indicated that 20 (13.4%) multidrug-resistant *K. pneumoniae* isolates possessed *intII* [33]. Moreover, our findings indicated that *IntI* was most frequent than *IntII*, consistent with observations from other investigations [34]. All

integron-positive bacteria, regardless of class, exhibited a multidrug-resistant phenotype. Our investigation found no connection among the abundance of Class 1 with/or 2 integrons and resistant to certain antibiotics classes. Numerous investigations have demonstrated that the existence of IntI was correlated with resistant to Aminoglycosides, Cephalosporins, Tetracycline, and Chloramphenicol, whereas *IntII* is connected only with resistance to tetracycline [13]. A different investigation indicated that the assessment of two classes of integrons utilizing PCR technique revealed that 46 (48%) as well as 40 (42%) isolates have class I and II integrons, correspondingly [35].

The outcomes for the present CRKP isolates especially concerning. A total of 10 out of 16 isolates exhibited MDR, while DNA testing identified combinations of double or triple carbapenemase genes (*IMP-1, IMP-2, NDM-1*, *OXA-48, VIM-1, KPC-1*). The class B β-lactamase *NDM-1* ha's recently generated considerable apprehension. Moreover, several investigations have recorded the identification of enterobacterial isolates that generate *NDM-1* throughout diverse global areas (36). We found 6 (or 37.5% of the total) carbapenemases that generate *NDM-1* along with *IMP-1* and *IMP-2.* The *KPC* gene was present in a single one of our samples. The majority of the isolates (80.9%) had the triple resistance gene combination *KPC/ NDM-1/ OXA-48*, according to a Saudi Arabian study, whereas a small percentage (19.04%) showed either double resistance (*KPC/OXA-48*) or triple resistance (*NDM-1/OXA-48)* [36].

One Turkish investigation found that 81.05 percent of CRKP isolates produced *OXA-48*, whereas 389.0 percent produced *NDM-1* [37]. The dissemination of the genes encoding these enzymes amongst Gram- -"negative bacteria has made them an important cause of resistance". In addition, there rre currently on clinically accessible inhibitors to block metallo-β-lactamase action (38). There was a strong association among the existence of integron genes and enhanced resistant to various antibiotics classes, and the findings of this investigation showed that Integron was very prevalent in *Klebsiella*, particularly among hospitalized patients. In the horizontal transfer of resistance to antibiotics, integrons play a role as transposable genetic elements.

4 CONCLUSIONS

The findings of this study provide strong evidence of the growing complexity and clinical significance of carbapenem-resistant Klebsiella pneumoniae in healthcare settings. The high prevalence of class I and class II integrons among the isolates reflects a substantial genetic capacity for capturing, integrating, and disseminating resistance determinants. This, combined with the detection of multiple carbapenemase-encoding genes particularly *blaIMP-1*, *blaNDM-1*, and *blaOXA-48* demonstrates the organism's increasing adaptability and its ability to develop multidrug-resistant and extensively drug-resistant phenotypes.

The coexistence of several resistance genes within the same isolates underscores the potential for rapid intra- and interspecies transmission, especially in environments with sustained antibiotic pressure. These results highlight a critical public health concern, as such genetically diverse and highly resistant strains significantly limit therapeutic options, increase the risk of treatment failure, and enhance the likelihood of outbreaks within hospitals.

Overall, the study emphasizes the urgent need for early detection, continuous molecular surveillance, and strict infection-control strategies to prevent the further spread of carbapenem- resistant *K. pneumoniae*. Continued monitoring and comprehensive genomic investigations are warranted to better understand their epidemiological dynamics and to support the development of targeted interventions.

ETHICAL APPROVAL

The research was done following the ethical guidelines derived from the Declaration of Helsinki. Prior to enlisting participants for the research, verbal and analytical agreement was gained from the patient. The Institutional Ethics Committee at the Diyala University approved the research protocol (Ref, 9888, date 20/1/2025).

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