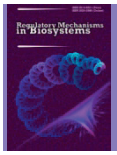

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

The molecular function of class A and B carbapenemase genes in the antibiotic resistance of *Klebsilla pneumoniae* isolated from urinary tract infection

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Article info

Received 15.03.2025
Received in revised form 26.04.2025
Accepted 20.05.2025

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Najim, A. H., & Ghrabi, W. A. (2025). The molecular function of class A and B carbapenemase genes in the antibiotic resistance of Klebsilla pneumoniae isolated from urinary tract infection. Regulatory Mechanisms in Biosystems, 16(2), e25077. doi:10.15421/0225077

Today, some of the β-lactamase enzymes that can render the last class of carbapenems inactive are the carbapenemase enzymes, which are derived from the bacterium *Klebsiella pneumoniae*. The purpose of this work is to identify and assess KPC and NDM-I gene expression in local isolates of *K. pneumoniae* using real-time PCR. In Baghdad City, Iraq, urine samples from patients with UTIs at Al-Yarmouk General Hospital and Al-Karama General Hospital yielded 150 *K. pneumoniae* isolates. Following cultivation on nutritional agar and MacConkey agar medium, *K. pneumoniae* was identified and the carbapenemase gene was detected using PCR. The presence of the *K. pneumoniae* 16S rRNA gene (95 bp), a gene used to identify *K. pneumoniae*, was detected molecularly using the polymerase chain reaction (PCR) for each extracted DNA sample. Specific primers were utilized. The findings of the traditional PCR method demonstrated that 20 (100%) of the 20 isolates of *K. pneumoniae* that were resistant to carbapenem carried the KPC gene, which has a size of 221 bp. These data show that our local isolates are resistant to carbapenem, which is associated with the production of the KPC gene. Twenty (100%) of the 20 carbapenem-resistant *K. pneumoniae* isolates were found to have the NDM1 gene (131 bp). Furthermore, in Real-Time PCR, all isolates treated with cranberry and bearberry herbal extracts exhibited down expression of the KPC and NDM1 genes in comparison to the housekeeping gene when compared to the control. Based on these findings, it can be concluded that the carbapenem-resistant isolates were members of the current class A and B genes, which are highly expressed in our local isolates.

Keywords: *Klebsellia*; carbapenems; real-time PCR; KPC gene; urinary tract infection.

Introduction

Although urinary tract infections (UTIs) are among the most prevalent infection types worldwide, comprehensive data on long-term trends is lacking. An estimated 404.61 million UTI infections occurred globally in 2019, leading to around 236,790 fatalities (Al-Samrraee, 2017; Yang et al., 2022). Acute cystitis (bladder infection), acute pyelonephritis (kidney infection), and silent bacteriuria are the three main categories into which UTIs are often divided. For the precise diagnosis and efficient treatment of UTIs, this categorization is essential (Mouanga et al., 2024). According to the Centers for Disease Control's (CDC) most recent data, antibiotic-resistant illnesses cause 35,000 fatalities and 2.8 million instances of infection in the US each year. Six AMR bacteria were identified as responsible for many deaths: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *K. pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus* (Mancuso et al., 2023; Mosa et al., 2022). One of the most common bacteria that causes urinary tract infections (UTIs) is *K. pneumoniae*. There are fewer treatment options available due to its growing resistance to a variety of medicines (Maraki et al., 2024). This made it necessary to monitor the local spread of resistant bacterial strains to manage infection in hospital settings using epidemiological approaches (Johnson, 2015; Mohamed & Al-Taai, 2023). Examining the isolates' molecular type and genotypic homology might help us understand bacterial transmission better, especially considering the spread of MDR *K. pneumoniae* in Iraqi hospitals (Akya et al., 2018; Al-Ameedi & Al-Rekabi, 2023), in addition to the origins or sources of bacteria (Azimi et al., 2019; Ayad et al., 2021). Molecular and genetic research methods can be used to examine the phylogenetic connections between different bacteria across different genomic areas. Different rRNA genes, including 16S rRNA, are often used in comparative studies to investigate the phylogenetic diversity of different bacterial species because of their high level of conservation and simplicity of amplification (Tshikhudo et al., 2013; Yang et al., 2016). This molecular method's primary advantages are its speed (the procedure may be completed in a matter of hours) and its ability to identify subtle species variations. Comparing the 16S rRNA gene sequence has emerged as the preferred genetic method for determining species differences and similarities. Advances in computer-aided methods for biological data processing further enable the quick and precise identification of bacterial species (Sedlar et al., 2017; Sadeq et al., 2024). Numerous research has used the 16S rRNA technique for bacterial identification in the context of uropathogens (Campos et al., 2018). *Klebsiella pneumoniae* carbapenemase (KPC) is a class-A β-lactamase, and the most active family of carbapenemases. The development of antibiotic resistance by class-A ambler enzymes, such as KPC, generally leads to increased cessation in the treatment of infections (Talebi & Hashemi, 2022). Detection of carbapenemases in Enterobacteriaceae is essential to control the development of resistance in this family, particularly in *K. pneumonia* isolates. The identification of *K. pneumonia* isolates producing KPC has become a major concern for clinicians (Bandari et al., 2022). NDM-producing isolates are usually transmitted through the movement of infected or colonized individuals between the original reservoir in the Indian subcontinent and the rest of the world, while the Middle East has been considered as a secondary reservoir (Wailan & Paterson, 2014). The same has also been attributed to the Balkan region, as reports from different European countries have documented infections due to NDM-1-possessing pathogens, following previous hospitalization in the Balkan states (Livermore et al., 2011). However, NDM-1- producing *K. pneumoniae* strains are only sporadically reported in that region, being occasionally documented from Balkan countries (Kocsis et al., 2016).

Materials and methods

The Ethics Committee of Al-Yarmouk, Al-Karama and Al-Musaib Teaching Hospitals in Baghdad and Babylon cities, Iraq approved the study protocol. Informed consent was obtained from each patient or their family member before participation. A total of 150 clinical specimens (UTI infection) were obtained from both sexes and a

range of age groups who were admitted to Al-Yarmouk General Hospital and Al-Karama General Hospital in Baghdad City, Iraq as well as Al-Musaib General Hospital in Babylon City, Iraq. The time frame for this investigation was August 2023–November 2024. Every urine sample was placed in a sterile, clean container. Prior to being incubated for 24 hours at 37 °C, urine samples were initially cultivated on nutritional agar and MacConkey agar medium.

To detect the 16S rRNA sequencing, pure bacterial cells (2×10^9 CFU/mL) cultivated in nutrient agar medium were extracted. The polymerase chain reaction (PCR) primers that were utilized to amplify the gene were Forward (GACGATCCCTAGCTGGTCTG) and Reverse (GTGCAATATTCCCACTGCT). According to manufacturing instructions as described by Cayci et al. (2019).

The strains were cultured for a full night at 37 °C in AB and LB media (5 mL), with each inoculum's optical density set to OD595, 0.08 nm. A SensiFAST SYBR NO-ROX Kit (BioLine, USA) was used to perform the qPCR reaction, and in accordance with the previously reported work (Atshan et al., 2013).

Out of 20 isolates of *K. pneumoniae* that were resistant to carbapenem, 20 (100%) of them carried the KPC gene, which has a size of 221 bp, according to the results of a traditional PCR approach (Fig. 2). These findings show that the carbapenem resistance of our local isolates is related to the production of the KPC gene. The NDM1-R gene (131 bp) PCR reaction data are displayed in Figure 3. Twenty (100%) of the twenty isolates of *K. pneumoniae* that were shown to be carbapenem-resistant had this gene.

Results

The specimens were classified according to age and gender of the carrier (Table 1). The results showed that out of 150 samples, 101 (67.4%) were from females while 49 (32.6%) were from male.s Furthermore the *K. pneumonia* appeared highly prevalent in females aged 21–30 years. 16S rRNA gene molecular identification in *K. pneumoniae*, to find *K. pneumoniae*, a polymerase chain reaction (PCR) was performed on each isolated DNA sample using particular primers. Figure 1 displays the outcomes of the 16S rRNA gene PCR process. The identification of 90 clinical samples as *K. pneumoniae* was verified.

The KPC gene, which has a size of 221 bp, was present in 20 (100%) of the 20 carbapenem-resistant *K. pneumoniae* isolates, according to the findings of a traditional PCR approach (Fig. 2). These data show that our local isolates are resistant to carbapenem, which is associated with the production of the KPC gene. Figure 3 displays the outcomes of the PCR reaction for the NDM1-R gene (131 bp). All twenty (100%) of the twenty carbapenem-resistant *K. pneumoniae* isolates that were found had this gene.

Table 1
Distribution of specimen collection from patients with UTI according to age and gender

Age, years	Male	%	Female	%	Total	%	χ^2	P-value
1–10	9	6.6	9	5.0	21	11.7	0.42	0.510
11–20	11	8.8	17	12.2	38	21.1	0.94	0.330
21–30	15	7.2	28	15.5	41	22.8	5.48	0.019
31–40	11	6.1	18	10.0	29	16.1	1.69	0.190
41–50	5	5.5	20	12.2	32	17.8	4.50	0.034
51–60	2	2.7	9	5.0	14	7.8	1.14	0.280
Total	N = 49 (32.6%)	32.6	N = 101 (61.7%)	67.4	150	100.0	–	–
Chi-square (χ^2)	14.08	–	30.70	–	39.80	–	–	–
P-value	0.029	–	0.0001	–	0.0001	–	–	–

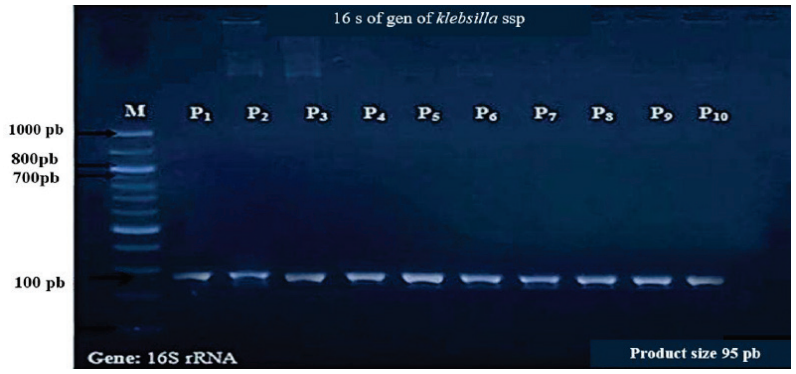

Fig. 1. PCR products and the 16S rRNA gene (95 bp) of *Klebsiella pneumoniae* isolates were separated using gel electrophoresis: lanes L and NC represent the 50 bp DNA ladder, negative control, and the 16S rRNA

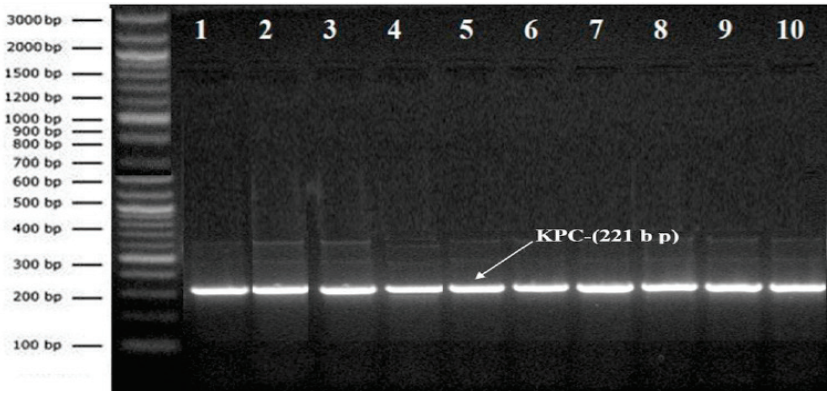

Fig. 2. PCR product of KPC gene (221 bp) of *K. pneumoniae* 100 bp, gel electrophoresis

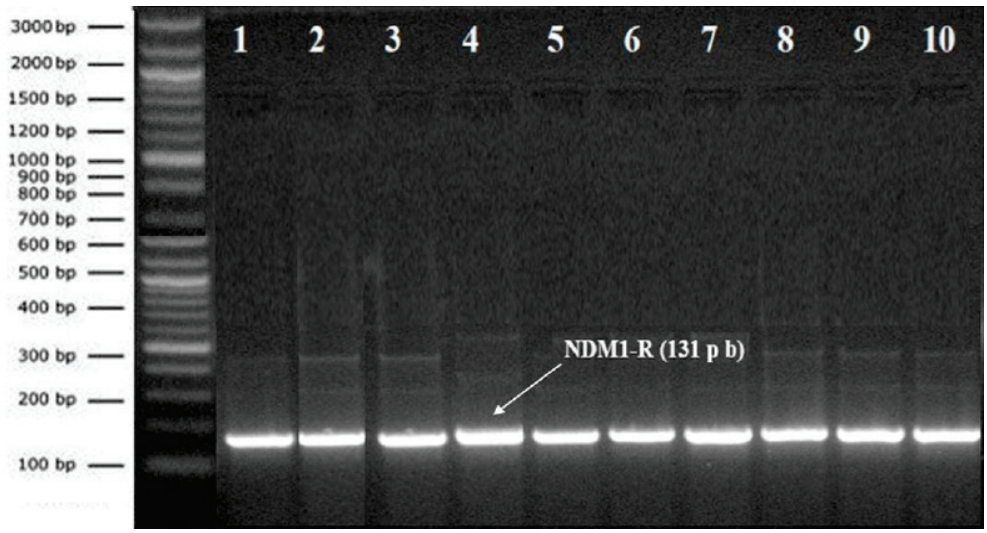

Fig. 3. The PCR product of the NDM1 gene (131 bp) of the *K. pneumoniae* 100 bp DNA ladder was electrophoresed on a gel

In this study, treatment and control samples of bacterial growth with bearberry and cranberry at concentrations below the minimum inhibitory concentration (Sub-MIC) for each sample were compared using a quantitative RT-PCR approach. The goal of the analysis was to ascertain the carbapenem genes' (KPC and NDM-1) mRNA expression. The Quantitative RT-PCR program was used to record the amplified genes' Ct values as illustrated in Figure 4. The relative quantification (RQ) method was used to calculate the gene expression fold change based on the delta Ct value as described in Figure 5. The results showed that the carbapenem genes (KPC and NDM-1) were significantly regulated in the local isolates. The bacterial isolates that tested against bearberry had carbapenem gene expression fold 0.0317, which was greater than the fold of the control (the fold change is 1) (Table 2). Additionally, the bacterial isolates that tested against cranberry showed gene expression fold (0.00895).This result was greater than the fold of the control (the fold change is 1) (Table 3).

Table 2
Expression level for bearberry in controls and treated samples

Group	Mean Ct target gene	Mean Ct housekeeping gene	Δ Ct	Δ Ct calibrator	ΔΔCt	$2^{-\Delta\Delta Ct}$	Fold change
Controls	15.68	27.41	11.72	11.72	0	0	1
Patients	16.28	21.11	4.83	11.72	6.89	0.0089	0.0089

Table 3
Expression level for cranberry in controls and treated samples

Group	Mean Ct target gene	Mean Ct housekeeping gene	Δ Ct	Δ Ct calibrator	ΔΔCt	$2^{-\Delta\Delta Ct}$	Fold change
Controls	24.34	22.06	2.28	2.28	0	0	1
Patients	31.11	23.91	7.20	2.28	4.92	0.0317	0.0317

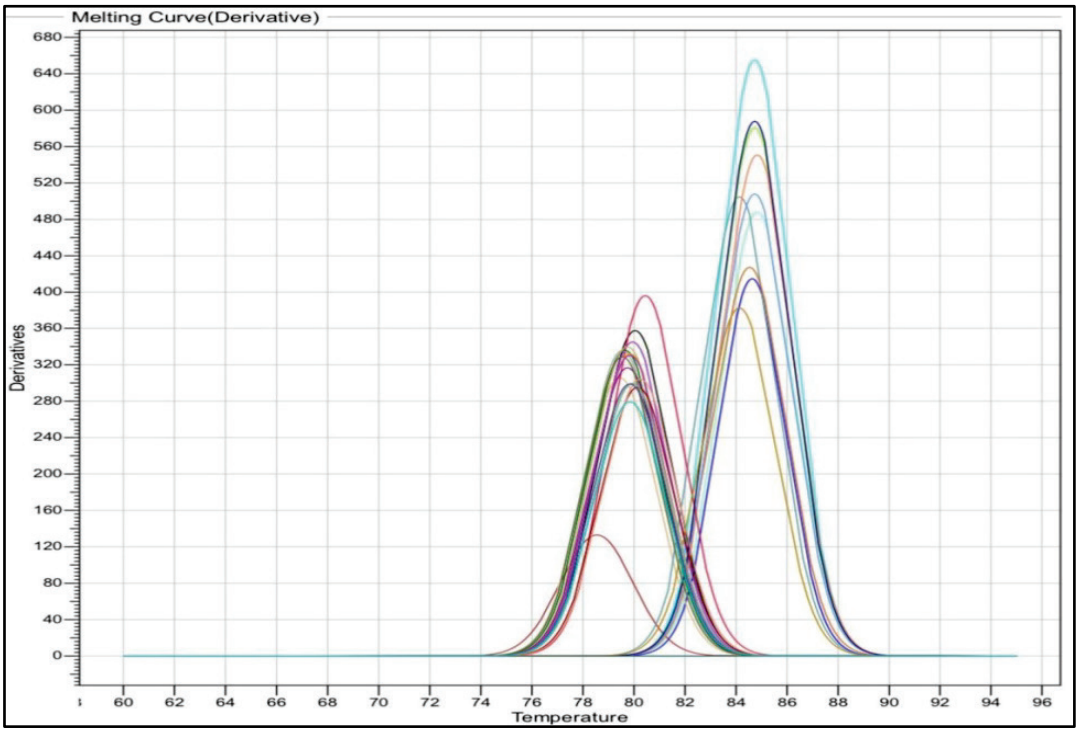

Fig. 4. Melting curve (derivative) NDM-1 and KPC by qPCR samples

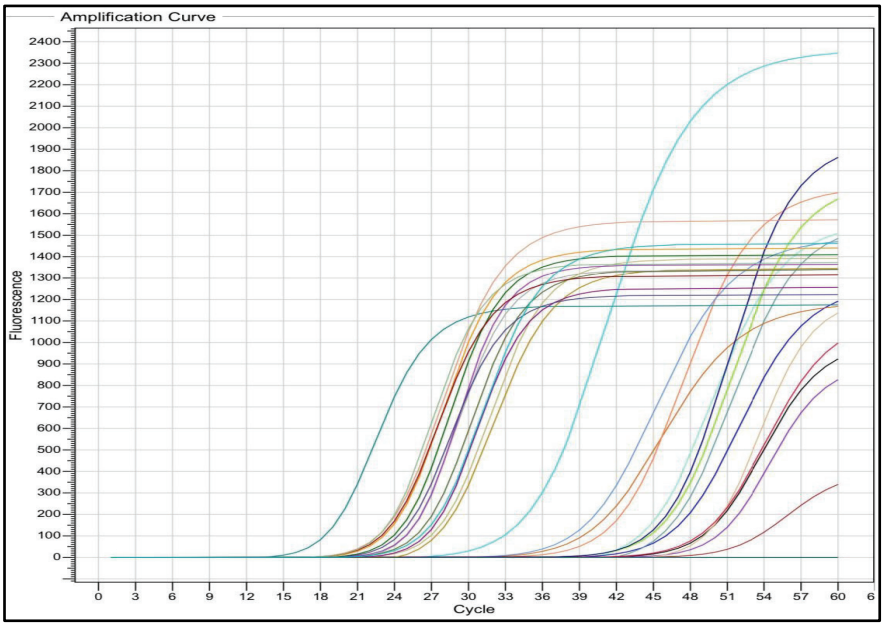

Fig. 5. Amplification curve of NDM-1 and KPC by qPCR samples

Discussion

The isolated samples were resistant to several drugs. Isolates can become resistant to new drugs more quickly than they can produce new antimicrobial compounds. Widespread resistance is a result of antibiotic misuse in medicine, agriculture, and animal husbandry. In clinical microbiology labs, *Klebsiella* is hard to find and frequently misclassified (Shankar et al., 2018). Many studies have been demonstrated to ascertain the effectiveness of various methods for identifying distinct *Klebsiella* species (Mukherjee et al., 2020). These studies show how a PCR technique works by using DNA polymerase to create a new DNA strand that is complementary to the template strand that is being provided. At the end of the PCR process, the chosen sequence will be put together in billions of copies (Amplicon) (Al-Dulaimi et al., 2022). By comparing the molecular weights of the bands that appeared on the electrophoresis gels to 100 bp DNA ladders, the PCR results were confirmed. According to Figure 1, every isolate in this investigation tested positive for the 16S RNA gene with 95 bp. Only *K. pneumoniae* possesses the 16srRNA gene, which has been shown to be helpful for molecular characterisation of the bacteria (Ghaima, 2018). The current findings resembled those of investigations conducted in Iraq by Ghaima (2022) and Aboud et al. (2022). The 16S rRNA primer gene for *K. pneumoniae* was amplified using DNA samples that were taken from suspected isolates and identified as such using standard PCR. The results corroborate those of earlier research showing that the 16srRNA gene may be detected as an extra tool to identify the organism at the genus level and the species level, as validated by other techniques (Cayci et al., 2019). Although recent investigations have shown high-level resistance to carbapenems, carbapenems are the most effective drugs against multiple antibiotic-resistant *K. pneumonia* (Auda et al., 2017; Kareem, 2020). Even though this type of resistance has been highly researched worldwide, research in Iraq is still lacking and does not fully address the extent of this serious issue, particularly concerning the antibiotic carbapenem's limited use in treating bacterial infections.

In Canada, Korea, Greece, Kenya, Italy, and Iraq, several outbreaks of KPC and NDMI-1 genes-producing *K. pneumoniae* have been documented; numerous studies indicated that the fatality rate in Greece was 37.0% and in Italy, 41.6% (Leavitt et al., 2007; Campos et al., 2016). The prevalence of resistance is in line with the sharp rise in producers' recurrence among clinical isolates in several global locations (Hussein et al., 2022). Globally, there is strong evidence of the steady rise in carbapenem-resistant strains, which means it is a life-threatening problem (Mohammed et al., 2020). There were significant differences in the mean fold change across the groups in the research. Since the mean Ct values in resistant bacteria following treatment were greater than those in resistant strains before meropenem treatment, the genes are found in mRNA samples (Rebouças et al., 2013). Following cranberry and bearberry therapy, these results demonstrate that the resistant group had the largest copy number of target genes carried on mRNA, indicating increased expression.

This study concurs with (Aboud et al., 2022). Because these isolates were resistant to these herbals, as indicated in Tables 1 and 2, comparing the gene expression values of KPC and NDMI genes in control and treated samples demonstrates the presence of expression among the examined genes. The findings of the study by Bogaerts et al. (2017), suggest that the resistance under investigation is caused by a variety of resistance mechanisms, including mutations, overproduction of ESBLs, and loss of porin. Additionally, overexpression of the KPC and NDMI-1 genes was seen in the RT-qPCR. These findings indicate that the development of these two genes is responsible for the resistance in Baghdad.

Conclusion

In every examined isolate, the class A and B carbapenem genes were detected (100%). At sub-inhibitory quantities of the antibacterial cranberry and bearberry, it was shown that the expression of two carbapenem genes was significantly downregulated. Additionally, when compared to control samples, the gene expression data for natural herbals such cranberry and bearberry extract showed minimal expression.

The authors express gratitude to the Institute of Genetic Engineering and Biotechnology, Baghdad University for their helpful provision and evidence in carrying out this research.

The author asserts that there is no conflict of attention in the publication of this object.

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