



Molecular Detection of NDM-1 and *ugpE* Genes in Multidrug-Resistant *Klebsiella pneumoniae* Isolates and Their Correlation with Antibiotic Resistance

Zahraa Redha Shamsee ^{1*}, Aida Hussain Ibrahim ¹

Abstract

Background: *Klebsiella pneumoniae* is ubiquitous gram-negative encapsulated pathogen that has long been associated with variety of infections worldwide. Antimicrobial resistance, particularly multidrug resistance (MDR), shows a serious threat to public health, with *K. pneumoniae* being one of the leading culprits. Mechanisms such as production of carbapenemases like NDM-1 and virulence factors like *ugpE* contribute to resistance and pathogenesis. The study aimed to assess the expression of NDM-1 and *ugpE* genes in *K. pneumoniae* isolates and their association with antibiotic resistance. **Method:** Clinical samples were collected, and *K. pneumoniae* isolates were identified using biochemical tests and molecular techniques. Antibiotic susceptibility testing was conducted using the disk diffusion method, and MIC values were determined for colistin and meropenem. DNA and RNA were extracted, and PCR was performed to detect NDM-1, *ugpE*, and 16S rRNA genes. Gene expression was evaluated using quantitative real-time PCR. **Results:** Among 210 clinical samples, 90 *K. pneumoniae* isolates were identified. Antibiotic

susceptibility testing revealed high resistance to multiple antibiotics, with vancomycin showing 100% resistance. PCR confirmed the presence of NDM-1 (23%) and *ugpE* (93%) genes. MIC results showed variable resistance patterns. Gene expression analysis demonstrated a significant decrease in NDM-1 expression and an increase in *ugpE* expression after colistin treatment, while meropenem treatment led to up-regulation of NDM-1. **Conclusion:** *K. pneumoniae* isolates exhibited high resistance to various antibiotics, with significant expression of NDM-1 and *ugpE* genes. Colistin treatment influenced gene expression more than meropenem, highlighting the importance of understanding resistance mechanisms for effective management of infections.

Keywords: *Klebsiella pneumoniae*, NDM-1 gene, *ugpE* gene, multidrug resistance, antibiotic susceptibility, gene expression

Introduction

Klebsiella pneumoniae is a versatile and opportunistic Gram-negative bacterium belonging to the Enterobacterales order, known for its ability to thrive in both aerobic and anaerobic environments. This pathogen can colonize the mucosal linings of the human gastrointestinal and nasopharyngeal tracts, resulting in a broad spectrum of infections, including sepsis, soft tissue infections, respiratory tract infections, urinary tract infections (UTIs), and liver abscesses (Khalaf and AL-Hashimy, 2022). *K. pneumoniae* is particularly notorious for its association with antimicrobial

Significance | *Klebsiella pneumoniae* showed a global threat due to its multidrug resistance. This study elucidated resistance mechanisms and antibiotic susceptibility, crucial for treatment strategies.

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resistance, earning its place among the ESKAPE pathogens—a group that includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species—due to their ability to "escape" the effects of many antibiotics (Al-Gbouri and Hamzah, 2018).

The alarming global prevalence of multidrug resistance (MDR) in *K. pneumoniae* represents a significant public health challenge, emphasizing the critical need for regular antimicrobial susceptibility testing to determine effective antibiotic treatments (Algammal et al., 2020). A key factor in the bacterium's resistance arsenal is its ability to produce enzymes such as carbapenemases, which degrade β -lactam antibiotics (El-Domany et al., 2021). Other resistance mechanisms include changes in outer membrane permeability, the production of extended-spectrum beta-lactamases (ESBLs), overexpression of AmpC enzymes, mutations in porins and penicillin-binding proteins (PBPs), metabolic pathway modifications, efflux pump activity, and biofilm formation capability (AL-Jubouri and Shami, 2022).

Carbapenems, a class of β -lactam antibiotics, are often employed as a last resort for treating severe infections caused by MDR Gram-negative bacteria, especially when other antibiotics, such as cephalosporins or those targeting ESBL-producing bacteria, prove ineffective (Mahmood, 2022). The discovery of the New Delhi Metallo- β -lactamase-1 (NDM-1) enzyme has further intensified global concerns, as it imparts resistance to almost all β -lactam antibiotics, posing a substantial threat to public health (Khan et al., 2017). Infections caused by NDM-1-producing bacteria are associated with increased risks of UTIs and other serious infections (Mochon et al., 2011).

In addition to these resistance mechanisms, certain chromosomal genes within the Ugp group, such as *ugpA*, *ugpB*, *ugpC*, and *ugpE*, are crucial for the uptake of sn-glycerol-3-phosphate. The bacterial ABC transporter system, which relies on a substrate-binding protein (SBP), is pivotal in transferring substrates to the membrane-bound transporter complex (Licht and Schneider, 2011).

Current research aims to investigate the expression levels of the NDM-1 and *ugpE* genes in *K. pneumoniae* isolates under the influence of the antibiotics meropenem and colistin. This is accomplished using Real-Time Quantitative PCR (RT-qPCR), a technique that allows for precise quantification of gene expression. Understanding these molecular mechanisms of resistance will aid in identifying potential therapeutic targets and devising effective interventions.

Masteries and Methods

Isolation and Identification of Bacterial Isolates:

Clinical samples were collected from multiple hospitals in Baghdad between March 2022 and July 2022, with ethical approval obtained from the Baghdad College of Science's ethics committee (CSEC/0523/0042). A total of 210 clinical samples were obtained from various sources including urinary tract infections (UTI), sputum, wound swabs, blood, and liver abscesses. Initial bacterial isolates were identified using selective and differential culture media, followed by biochemical testing and confirmation using the Vitek-2 system. Molecular identification was further validated using the 16S rRNA gene (Omar and Ibrahim, 2023).

Antibiotic Susceptibility Test:

Antibiotic susceptibility testing was performed via the disk diffusion method. The following antibiotics were tested: ampicillin (AMP, 10 μ g), amoxicillin-clavulanate (AMC, 10/20 μ g), ceftazidime (CAZ, 30 μ g), cefixime (CFM, 5 μ g), streptomycin (STR, 10 μ g), tetracycline (TET, 30 μ g), imipenem (IMP, 10 μ g), doripenem (DOR, 10 μ g), vancomycin (VA, 30 μ g), nalidixic acid (NAL, 30 μ g), ciprofloxacin (CIP, 5 μ g), chloramphenicol (CHL, 30 μ g), aztreonam (ATM, 30 μ g), cefotaxime (CTX, 30 μ g), nitrofurantoin (NIT, 300 μ g), trimethoprim (TMP, 5 μ g), tobramycin (TOB, 10 μ g), trimethoprim-sulfamethoxazole (STX/ST, 10/20 μ g), and meropenem (MEM, 10 μ g). For colistin (CST, 10 μ g) and polymyxin B (PMB, 300 μ g), the minimum inhibitory concentration (MIC) method was employed. Results were interpreted according to Clinical Laboratory Standards Institute (CLSI, 2022) guidelines.

Molecular Study:

Extraction of DNA:

DNA extraction from *K. pneumoniae* isolates was performed using the Monarch[®] Genomic DNA Purification Kit (NEB, England).

Estimation of DNA Concentration:

The concentration of extracted DNA was quantified using the Qubit 4.0 Fluorometer (Invitrogen, USA).

Primer Design:

Primers specific for detecting NDM-1 and *ugpE* genes in *K. pneumoniae* were designed, ensuring optimal binding sites and annealing temperatures were verified using tools such as the Oligo Analyzer by IDT. Primer sequences and amplicon sizes are detailed in Table 1.

Polymerase Chain Reaction (PCR):

PCR detected *K. pneumoniae* and target genes (NDM-1 and *ugpE*). The reaction conditions included initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 35 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute. PCR products were visualized using 2% agarose gel containing RedSafe dye and UV light.

Minimum Inhibitory Concentration (MIC):

MIC values for colistin and meropenem against three selected antibiotic-resistant *K. pneumoniae* isolates were determined using

the micro-titer plate dilution technique in Mueller-Hinton broth, following CLSI (2020) guidelines.

RNA Extraction:

For gene expression analysis of NDM-1 and *ugpE*, RNA was extracted from *K. pneumoniae* using the TRIzol™ RNA Purification Kit (ThermoFisher, USA).

cDNA Synthesis:

Complementary DNA (cDNA) was synthesized from mRNA using the Luna Universal qPCR Master Mix Kit (NEB Company, UK). The process involved initial denaturation at 95°C for 10 minutes, annealing at 42°C for 30 minutes, and a final extension at 85°C for 5 minutes.

Quantitative Real-Time PCR (qPCR):

Gene expression levels were assessed using qPCR for NDM-1 and *ugpE* genes in three selected *K. pneumoniae* isolates. SYBR Green dye was used for fluorescence detection on a Bioer real-time quantitative PCR system (Japan). The protocol included initial denaturation at 95°C for 8 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 40 minutes. The Livak equation determined relative gene expression normalized to the housekeeping gene 16S rRNA.

Statistical Analysis:

Data analysis was performed using the Statistical Analysis System (SAS, Cary, 2012). The Least Significant Difference (LSD) test (ANOVA) and Chi-square test were employed to compare means and percentages at significance levels of 0.05 and 0.01.

Results and Discussion

Isolation and Identification of *K. pneumoniae*:

Two hundred ten clinical specimens were collected from various sources, including urine, sputum, wound swabs, blood, and liver abscesses, yielding 90 isolates identified as *Klebsiella pneumoniae* after incubation on MacConkey agar, blood agar, and CHROM agar media. The characteristic colony morphologies observed on these media confirmed the identification of *K. pneumoniae*. On MacConkey agar, isolates exhibited mucoid, pink-colored colonies due to lactose fermentation and capsule production, consistent with previous reports (Saleh et al., 2023). Colonies on blood agar appeared large, white-gray, and mucoid without hemolysis, while CHROM agar facilitated identification with metallic blue colonies specific to *K. pneumoniae* (Aboud et al., 2022).

Biochemical tests further validated the identity of isolates, showing positive results for urease, citrate utilization, catalase, and Voges-Proskauer, and negative results for oxidase, indole, and methyl red tests. Confirmation using the Vitek 2 System solidified the identification of *K. pneumoniae*.

Distribution of *K. pneumoniae* Isolates:

The distribution analysis (Table 2) revealed that urinary tract infections (UTIs) accounted for 57.7% of isolates, followed by sputum (24.4%), blood (10%), wound (5.5%), and liver abscesses (2.2%). These findings are consistent with previous studies conducted in different regions, demonstrating similar prevalence rates in urine and blood samples (Saadatian et al., 2018; Al-Ruobayiee and Ibrahim, 2023; Rhumaid and Al-Mathkhury, 2015).

Antibiotic Susceptibility Testing:

Ninety *K. pneumoniae* isolates underwent antibiotic susceptibility testing using the disk diffusion method against 21 antibiotics. The results (Figure 2) indicated varying resistance profiles, with the highest resistance observed against Vancomycin (100%), Ampicillin (97.77%), Amoxicillin-clavulanate (96.66%), Cefixime (93.33%), and Cefotaxime (93.33%). Conversely, lower resistance was observed against Colistin (25.55%) and Meropenem (31.11%). These findings corroborate regional studies highlighting high resistance rates to Ampicillin, Cefotaxime, and other β -lactam antibiotics (Albadri et al., 2021). The notable sensitivity to Colistin aligns with reports indicating its efficacy against carbapenem-resistant strains, making it a critical treatment option in such cases (Abdelhamid et al., 2021; Hamad, 2022).

Mechanisms of Antibiotic Resistance:

Multidrug resistance mechanisms in *K. pneumoniae* involve various factors such as mutations, enzymatic degradation (e.g., carbapenemase activity), alterations in bacterial porins, drug inactivation, and increased efflux activity (Singh et al., 2015). These mechanisms contribute to the widespread resistance observed across different antibiotic classes, emphasizing the complexity of combating infections caused by multidrug-resistant pathogens.

Molecular Detection:

16S rRNA Gene Detection:

The 16S rRNA gene was detected using PCR, confirming the presence of *K. pneumoniae* in clinical isolates. The positive results (Figure 3) demonstrated a molecular size of 172 bp, consistent with previous studies utilizing this gene for species identification (Ghaima and Tamara, 2022).

Detection of NDM-1 and *ugpE* Genes:

PCR analysis targeted the carbapenemase gene NDM-1 and the virulence gene *ugpE* in 80 MDR isolates. Results indicated a prevalence of 23% for NDM-1 and 93% for *ugpE* among *K. pneumoniae* isolates. These findings align with global and regional studies highlighting the role of NDM-1 in β -lactam resistance and the ubiquitous presence of *ugpE* in Enterobacterales members (Farzana et al., 2013; Rahman et al., 2014; Hussein, 2018; Yıldız et al., 2017). The statistically significant difference in gene frequency across infection sources underscores the importance of understanding gene distribution in clinical settings ($p=0.0064$ for NDM-1 and $p=0.0001$ for *ugpE*).

Minimum Inhibitory Concentration (MIC) Results:

Table 1. The oligonucleotide designed primers used in PCR amplification

Primers	Sequence (5'-3')	Amplicon size (bp)	Reference
16S RNA	F: CGGTCTGTCAAGTCGGATGT R: AGCGTCAGTCTTTGTCCAGG	172bp	Designed in this study
NDM-1	F: GCATTAGCCGCTGCATTGAT R: TAGGAAGTGTGCTGCCAGAC	146bp	
UgpE	F: CCTGGGTATCGCCGTCATTT R: AGCATCATCAGCCAGAACGG	183bp	

Table 2. The number and percentage of *K. pneumoniae* isolated from different sources

Types of Samples	Total Samples N.O	Bacterial strain N.O	Percentage (%*)	Percentage (%**)
Urine	105	52	50%	57.7%
Sputum	41	22	19.5%	24.4 %
Wound	31	5	14.7%	5.5%
Blood	27	9	12.8%	10%
Liver abscess	6	2	2.8%	2.2%
Total	210	90	100%	100%
P-value	---	---	0.0001 **	0.0001 **

** (P≤0.01).

Percentage (%*) distribution of *K. pneumoniae* relative to the clinical samples, Percentage (%**) distribution of *K. pneumoniae* relative to total no. of *K. pneumoniae* strain.

Table 3 The prevalence of genes among *K. pneumoniae* strains isolated from various clinical sources

Samples	Urine (46)	Sputum (19)	Wound (4)	Blood (9)	liver abscess (2)	P-value
NDM-1 (%)	10 (21.7%)	4 (21%)	1 (25%)	3 (33.3%)	0	0.0064 **
ugpE (%)	39 (84.7%)	19 (100%)	4 (100%)	9 (100%)	2 (100%)	0.0001 **

** (P≤0.01)

Table 4 MIC values (µg/ml) for three selected *K. pneumoniae* isolates with two antibiotics using the microtiter plate dilution method

<i>K. pneumoniae</i> isolates	MIC of Colistin	Sub-MIC of Colistin	MIC of Meropenem	Sub-MIC of Meropenem
3	4	2	64	32
11	4	2	128	64
43	8	4	8	4
P-value	0.0492 *	0.139 NS	0.0001 **	0.0001 **

** (P≤0.01), NS: Non-Significant



Figure 1. Colonies of *K. pneumoniae* on: A: Macconkey agar, B: blood agar C: CHROMagar after 24 hours of incubation at 37 C.

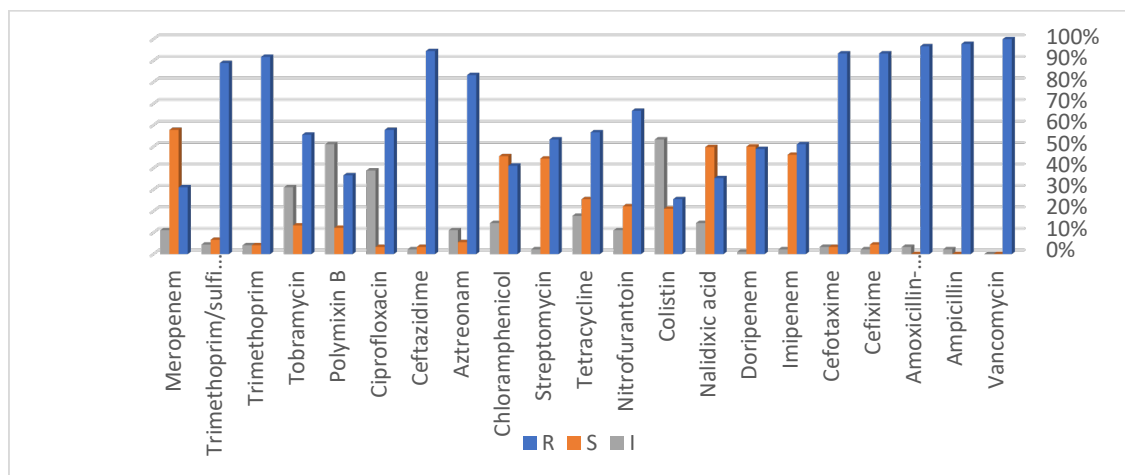


Figure 2. Antibiotic resistance test of *Klebsiella pneumoniae*

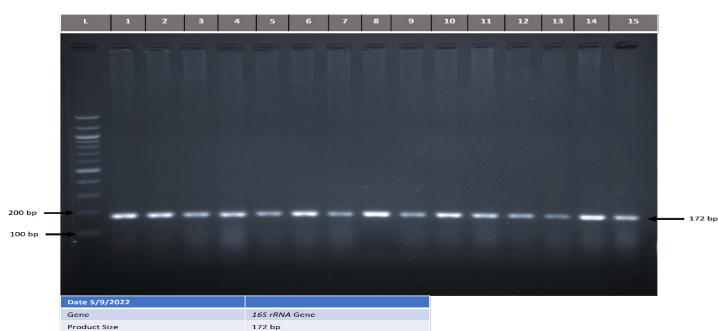


Figure 3. Gel electrophoresis of 16S rRNA gene (172bp) amplification for *K. pneumoniae*. Lane L: 100-200 bp DNA ladder; Lanes 1-15: *K. pneumoniae* isolates positive. (agarose 2%,80V for 80 min.).

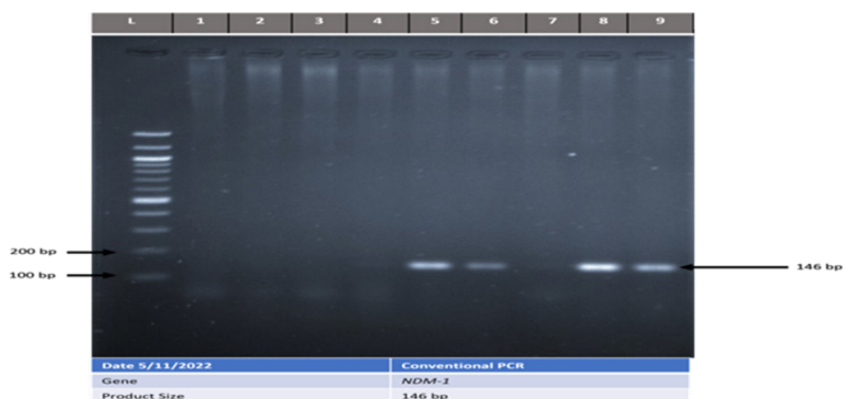


Figure 4. Agarose gel electrophoresis of PCR products for the NDM-1 gene (146bp). Lane M: 100-200 bp DNA ladder; Lanes 1-9: *K. pneumoniae* isolates. (agarose 2%,80V for 80 min.).

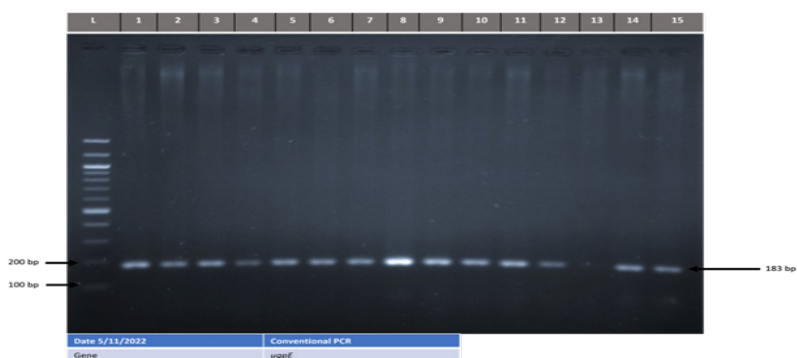


Figure 5. Agarose gel electrophoresis of PCR products for *ugpE* gene (183bp). Lane M: 100-200 bp DNA ladder; Lanes 1-15: *K. pneumoniae* isolates. (agarose 2%,80V for 80 min.).

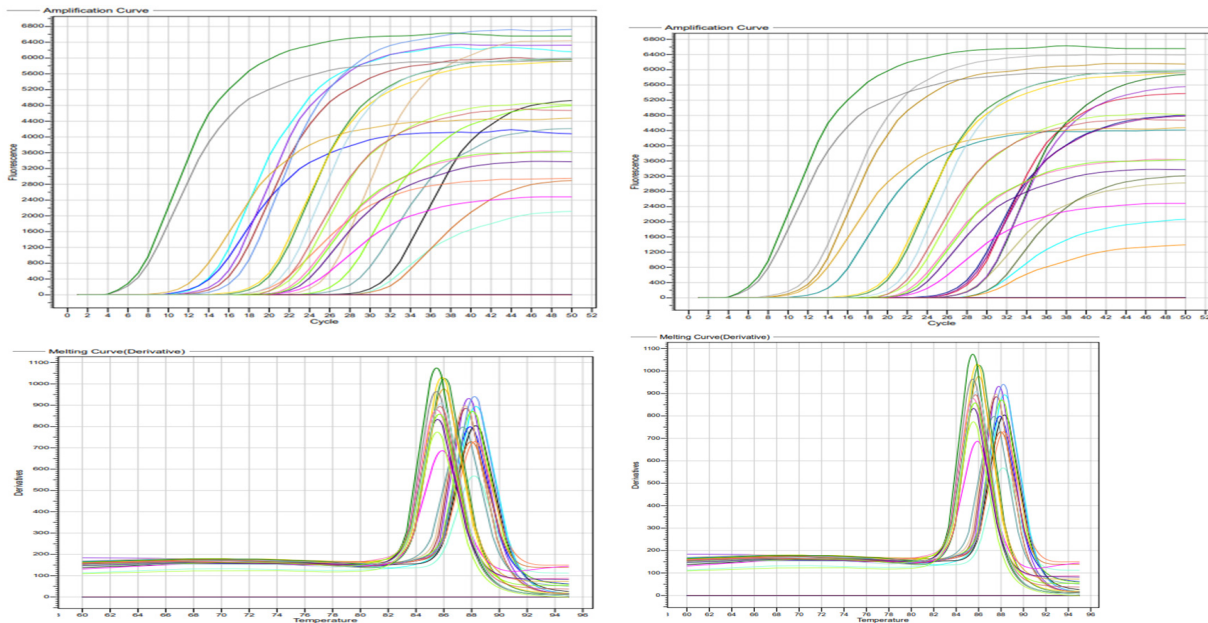


Figure 6. The cycling threshold of *K. pneumoniae* of the NDM-1 and *ugpE* genes as determined by real-time PCR and the image captured at the end of the RT-qPCR program; the melting curves of the NDM-1 and *ugpE* genes.

MIC testing of selected isolates resistant to multiple antibiotics showed colistin MICs ranging from 4-8 µl and meropenem MICs ranging from 8-128 µl. These results indicate bactericidal activity against colistin-resistant strains and variable resistance patterns to meropenem, consistent with previous findings (Kulengowski et al., 2017; Gautam and Tomar, 2022).

Gene Expression using RT-PCR:

Quantitative RT-PCR assessed the expression of NDM-1 and *ugpE* genes post-antibiotic exposure. Results indicated significant changes in gene expression levels (p-value = 0.0001 for NDM-1; p-value = 0.0028 for *ugpE*), with decreased expression of both genes following colistin treatment. Conversely, meropenem exposure resulted in up-regulated NDM-1 expression, highlighting its association with carbapenem resistance mechanisms (Poirel et al., 2011; Hamad, 2022). The findings underscore the complex interplay between antibiotic pressure and gene expression in promoting resistance.

This study provides comprehensive insights into the epidemiology, antibiotic susceptibility profiles, and molecular characteristics of *K. pneumoniae* isolates from clinical specimens. The prevalence of multidrug-resistant strains and the detection of key resistance genes and their expression patterns underscores the urgent need for effective antimicrobial stewardship practices. Future research should focus on continuously surveilling resistance mechanisms and developing novel therapeutic strategies to combat infections caused by multidrug-resistant *K. pneumoniae*.

Conclusion

This study underscores the concerning levels of antibiotic resistance observed among *Klebsiella pneumoniae* isolates, which have profound implications for clinical practice. Vancomycin resistance was universally detected across all isolates, emphasizing the critical shortage of effective treatment options against infections caused by these pathogens. In contrast, colistin demonstrated the lowest resistance rate at 25.55%, positioning it as a promising therapeutic choice for combating multidrug-resistant strains of *K. pneumoniae*. Molecular analysis using PCR revealed a prevalent presence of the *ugpE* gene (93%) and the NDM-1 gene (23%) among the isolates studied. These findings highlight the diverse genetic mechanisms contributing to antibiotic resistance in *K. pneumoniae*, with *ugpE* associated with virulence and NDM-1 linked to carbapenem resistance.

Furthermore, gene expression analysis through RT-PCR demonstrated significant changes in bacterial isolates following treatment with colistin compared to meropenem. Colistin treatment was associated with reduced expression of both NDM-1 and *ugpE* genes, suggesting effective suppression of resistant phenotypes under colistin pressure. In contrast, meropenem exposure led to up-regulated NDM-1 expression, indicating

adaptive responses to carbapenem exposure and illustrating the complexity of antibiotic resistance mechanisms in *K. pneumoniae*. In conclusion, these findings underscore the urgent need for enhanced surveillance strategies and antimicrobial stewardship practices to curb the spread of multidrug-resistant *K. pneumoniae* strains. Future research should prioritize the development of innovative therapeutic approaches and exploring alternative treatment modalities to effectively address infections caused by these challenging pathogens.

Author contributions

Z.R.S., A.H.I. conceptualized, analyzed data, prepared manuscript.

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Competing financial interests

The authors have no conflict of interest.

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